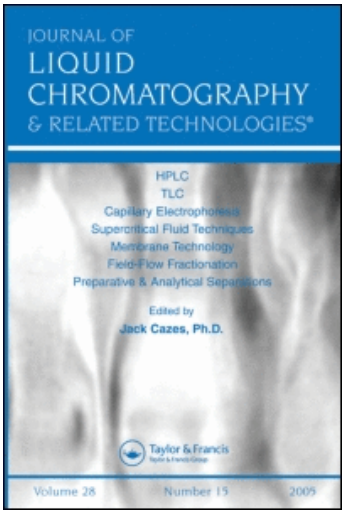


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Quantitative Analysis of a Chloroquinolin-Ethenyl Phenyl Derivative Using Thin Layers Impregnated With Di-P-Toluyl Tartaricacid

K. Price[a]; H. Perpall[a]; G. Bicker[a]; P. Tway[a]; N. Grinberg[a]
[a] Merck Sharp & Dohme Research Laboratories Rahway, New Jersey

To cite this Article Price, K. , Perpall, H. , Bicker, G. , Tway, P. and Grinberg, N.(1990) 'Quantitative Analysis of a Chloroquinolin-Ethenyl Phenyl Derivative Using Thin Layers Impregnated With Di-P-Toluyl Tartaricacid', Journal of Liquid Chromatography & Related Technologies, 13: 14, 2783 — 2807
To link to this Article: DOI: 10.1080/01483919008049071
URL: http://dx.doi.org/10.1080/01483919008049071

JOURNAL OF LIQUID CHROMATOGRAPHY, 13(14), 2783-2807 (1990)

QUANTITATIVE ANALYSIS OF A CHLOROQUINOLIN-ETHENYL PHENYL DERIVATIVE USING THIN LAYERS IMPREGNATED WITH DI-P-TOLUYL TARTARIC ACID

K. PRICE, H. PERPALL, G. BICKER, P. TWAY, AND N. GRINBERG*
Merck Sharp & Dohme Research Laboratories
Rahway, New Jersey

ABSTRACT

Thin Layer Chromatography (TLC) is an established method for the evaluation of final product drug and intermediate impurity profiles. Quantitative TLC has gained credibility within the Pharmaceutical Industry as a result of the latest developments in an availability of scanning technology. In the present paper we wish to report a quantitative TLC method for the determination of some potential impurities which may exist in a final bulk drug MK0679. In order to improve the selectivity of the chromatographic method, di-p-toluyl tartaric acid was impregnated on the stationary phase. Utilizing the modified layer, complete separation of the known impurity was obtained. The calibration curves for all components studied were linear and the detection limits obtained were less than 5ng.

* Author to whom correspondence should be addressed.

INTRODUCTION

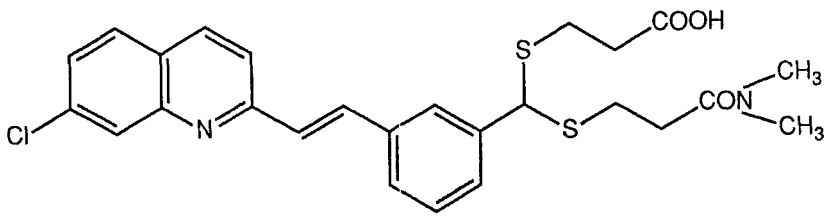
Thin layer chromatography (TLC) is one of the most popular techniques among chromatographic methods. It is not surprising that the number of publications on TLC has increased tremendously in last decade. Recent important developments in the field of TLC have been the emergence of new chemically bonded stationary phases along with the decrease in particle size [1,2]. Through these developments, improved efficiency and selectivity of the TLC plate have resulted. Ultimately applications which would otherwise have been difficult to perform have been achieved.

The advantages of TLC as a separation technique are its simplicity, ease of handling a large number of samples in a minimum amount of time, wide range of detection modes, and the wide selection of mobile phase solvents regardless of their UV absorbance. However, one of the biggest advantages of TLC is the ability to control the stationary phase selectivity by physically adsorbing into the TLC layer compounds which will further interact with the solute [3]. Interactions such as metal chelate complexing agents [4,5,6], π-π complexes [7,8], ion pairs [9,10], and ion exchange complexes [11,12] are only some of the

possibilities which can be used in order to improve the selectivity of a chromatographic system. There are an infinite number of possibilities by which layers can be modified.

In the field of Pharmaceutical Analysis, the place of TLC is well established among other analytical techniques [13]. Starting with raw material and ending with the final bulk drug, the impurity profiling of all components is critical. TLC can contribute to the accomplishment of that goal. Quantitative TLC has gained more and more credibility within the Pharmaceutical Industry especially with the latest developments in and availability of scanning technology.

Aryl and alkyl dithioacetals of mercaptoproprionic acid derivatives are potent receptor antagonists of leukotriene D_4 (LTD_4) and are being developed as therapeutic agents for bronchial diseases [14a, b]. One of the most promising candidates currently under development is MK0679. It is a potent orally active specific LTD_4 antagonist. Its potentially important role in the etiology of human asthma and other diseases suggest that



MK 0679

Figure 1. Chemical structure of MK0679.

leukotriene antagonists will offer effective new therapy [14c].

We wish to present in this paper a quantitative TLC method for the determination of some potential impurities which may exist in the MK0679 bulk drug (Figure 1).

In order to improve the selectivity of the chromatographic method, Di-p-toluyl tartaric acid was impregnated on the stationary phase. Utilizing the modified layer, complete separation of the known impurities was obtained. The TLC procedure has been developed and validated for the separation and weight percent determination of the known potential impurities of final product MK0679.

EXPERIMENTAL

Stationary Phase

HPTLC cyano plates (10 x 10 cm) containing fluorescence indicator at 250 nm were purchased from E. Merck (Darmstadt, Germany).

Mobile Phase

The mobile phase used in the experiments consisted of ethyl acetate-toluene-trifluoroethanol-triethylamine (75:10:5:10, v/v) with varied amounts of (-)-Di-O,O'-p-toluyl-L-tartaric acid (PTTA).

Chromatographic Chamber

The chromatographic experiments were performed in a rectangular twin trough chamber, 10 x 10 cm (Camag, Muttenz/Switzerland). The chamber was lined with a filter paper. The filter paper was wetted with the mobile phase in order to insure a saturated chamber atmosphere. The chromatographic chamber was considered equilibrated after 30 minutes with the mobile phase vapors. The level of the mobile phase in the tank was approximately 0.5 cm.

Sample Preparation

Samples were dissolved in methanol at 0.5 mg/ml. A synthetic mixture of MK0679 and known impurities was also prepared. This synthetic mixture was used as a system suitability sample.

Spotting and Development

Before spotting, the TLC plate was dipped into a solution of mobile phase until the plate was wetted homogeneously. The plate was then dried in a ventilated hood (1 - 2 hours) at room temperature. When the plate was dry, PTTA remained impregnated in the stationary phase. The sample solutions were then applied at the origin (1.5 cm from the plate bottom) by means of an automated spotter (Linomat IV, Camag, Muttenz/Switzerland). In order to avoid edge diffusion effects, the first and the last spots on the plate were positioned at a distance of at least 1 cm from the lateral edges of the plate. The plate was developed 8 cm from the origin. Once the separation was complete, the plate was removed from the chromatographic chamber and dried under a stream of nitrogen.

Detection

The detection was performed by fluorescence using a TLC scanner (Camag TLC Scanner II, Camag, Muttenz/Switzerland). The excitation was performed at 366 nm. A cut-off filter allowed for the detection of fluorescence emission above 400 nm. An HP 9000 computer model 300 equipped with Camag Evaluation Software 86 was used for all data treatment and integration. In order to establish the linearity of detection, a series of solutions of the known impurities were spotted. The amount of material spotted on the TLC plate ranged between 0.02 and 0.2 μg/spot. A calibration curve was obtained for each impurity. The statistics were calculated using Sigma-Plot and RS/1 software. For identification purpose in the case of an unknown sample, the TLC plates were scanned in the UV at 320 nm where PTTA does not absorb. At each spot maximum a spectra was taken.

RESULTS AND DISCUSSION

The stereochemical structure of MK0679 is presented in Figure 2.

The structure of the compound suggests that under initiating conditions (e.g. light) a radical can be

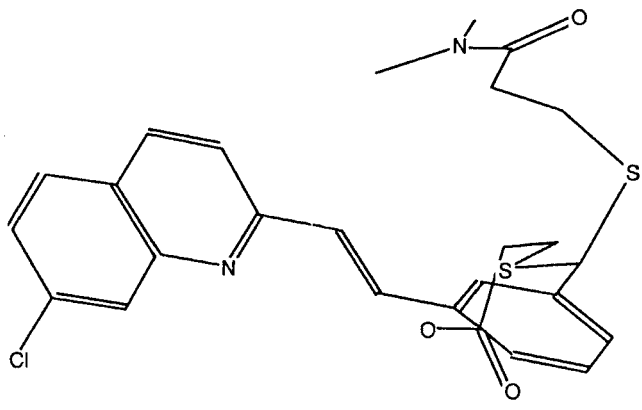


Figure 2. Stereochemical structure of MK0679.

formed which would further lead to a cis-trans isomerization around the double bond (Figure 3).

Previous NMR studies [15] indicate that a solution of the compound stored in clear glass vessels undergoes cis-trans isomerization. Through this study the equilibrium isomer concentration was determined to be 85% cis and 15% trans. This process may be of particular interest for the development of a chromatographic analytical method for this compound. The cis-trans isomerization was studied for the optimization of both the experimental conditions and the chromatographic resolution. Careful evaluation of the isomerization as a function of the experimental conditions has been completed. Chromatographic

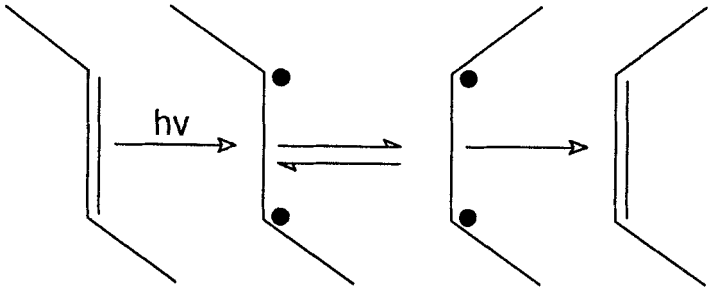


Figure 3. The reaction pathway for the cis-trans isomerization.

conditions were optimized based upon the separation of the cis and trans isomers

In reviewing the structure of MK0679, several functionalities exist which under normal phase chromatography on a silica stationary phase will result in strong solute-stationary phase interaction. To move this compound from the origin, a highly polar mobile phase would be required. These conditions may lead to compound decomposition. Therefore, cyano bond TLC plates were selected for the development of the method utilizing the dipole-dipole interaction between the solute and the cyano stationary phase.

The TLC method was initially developed using a mobile phase composed of ethyl acetate-toluene-

trifluoroethanol-triethylamine (75:10:5:10, v/v). Under these conditions, the interaction occurring between the stationary/mobile phase and the solute were not selective enough to resolve the geometrical isomers. In order to improve the selectivity of the method, the TLC plates were impregnated with (-)-Di-O,O'-p-toluyl-L-tartaric acid (PTTA). A mobile phase containing PTTA was prepared. A portion of the mobile phase was used for submerging and impregnating the plate while the other part was used for the chromatographic development. In this way, washing of PTTA from the layer during development was avoided. Dipping the plate yielded a PTTA impregnated surface. The PTTA is immobilized on the surface via dipole-dipole interaction with the cyano groups of the stationary phase. The modified PTTA stationary phase then interacts (under the mobile phase conditions) with the solutes via π-π interactions and hydrogen bonding leading to better selectivity and separation between the cis and trans isomers.

Several concentrations of PTTA in the mobile phase were studied to optimize the experimental conditions. The chromatograms obtained from the PTTA concentration study were scanned and the resolution

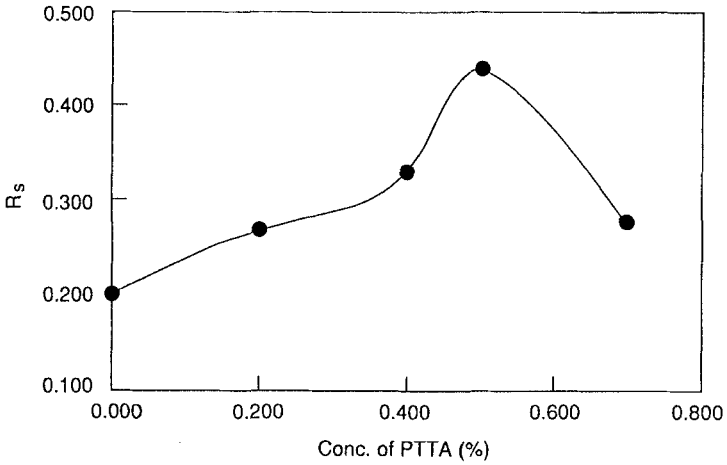


Figure 4. Effect of PTTA concentration on the resolution of cis and trans isomers. For chromatographic conditions see text.

between the two adjacent peaks (cis and trans) were calculated. Indeed, a level of 0.5% PTTA in the mobile phase produced the maximum in the resolution between cis and trans isomer (Figure 4).

Above this concentration of PTTA, the resolution begins to decline. This may be caused by the intermolecular interaction of PTTA molecules due to its high density in the stationary phase. Thus, the interaction between solute stationary phase will be minimized and the resolution will diminish.

A major problem which arises when dealing with a new compound is whether or not the extraneous spots

seen on the chromatogram are real or are artifacts of the system. In a previous study [16], some of the potential causes of such phenomena were reviewed. Since previous solution studies [15] showed that under light conditions the compound undergoes cis/trans isomerization, an investigation of experimental conditions in which cis and other degradates may be generated was completed. This study evaluated the effects of sample exposure to light during the sample preparation, spotting and development of the plate. In the initial experiment, the separation was completed entirely under laboratory light. In the second experiment, the sample was kept in the low actinic glassware. The spotting was completed under dark conditions, while the development of the TLC plate was performed under laboratory light. In the third experiment, the sample was kept in low actinic glassware, with both the spotting and the development of the plate completed under dark conditions. For each set of conditions, six spots, equal in amount, were spotted on the plate sequentially at ten minute intervals. The time elapsed between spotting of the first and the last spot was 60 minutes. The results presented in Figure 5a demonstrate that under total laboratory light conditions, the amount of cis isomer (R_f = 0.42) generated increased proportionally with

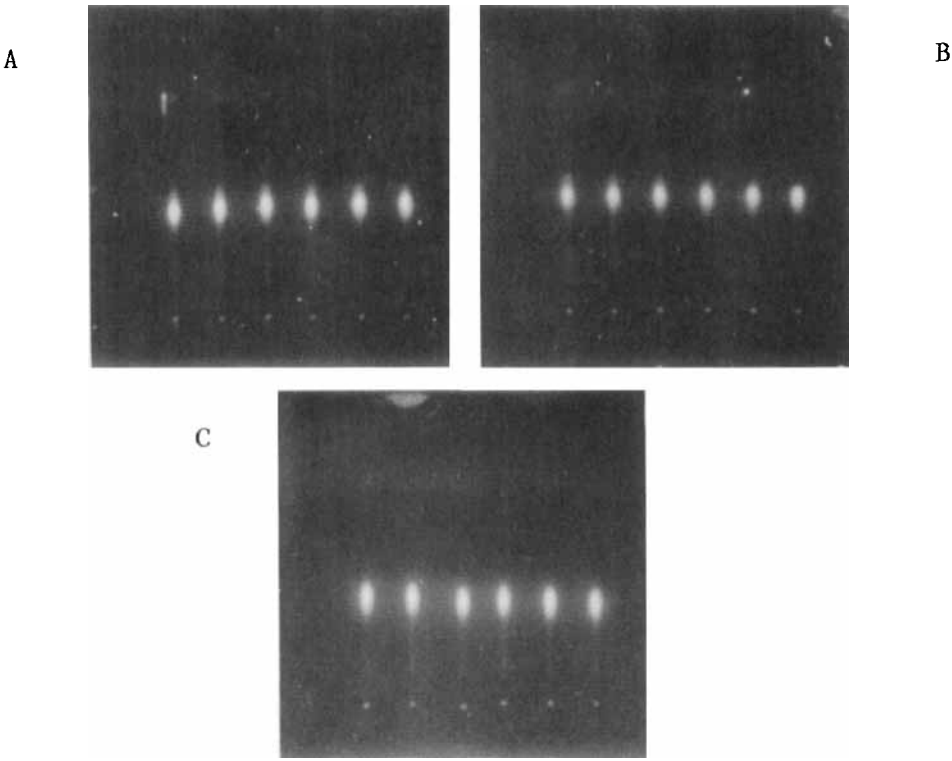


Figure 5. Dynamics of on-plate cis-trans isomerization. A - sample in clear/white glassware, syringe and chromatographic chamber exposed to light. B - sample in low actinic glassware, spotting and development under light conditions. C - sample in low actinic glassware, spotting and development under dark conditions. For chromatographic conditions see text.

the amount of time the material was allowed to sit on the plate before development.

At the same time, a new front running spot (R_f = 0.75) appeared following the same trend. In Figure 5b the results are presented for the experiment where the

Table 1

Dynamics of Cis/Trans Isomerization for MK0679*

Time	Trans (%)			Cis (%)		
(min.)	LApex	LAlight	CGlight	LApex	LAlight	CGLight
0	100.0	98.5	97.0	0	1.5	3.0
10	97.0	97.0	96.1	3.0	3.0	3.9
20	96.0	96.6	95.8	4.0	3.4	4.2
30	95.0	95.6	94.9	5.0	4.4	5.1
40	94.0	94.7	94.9	6.0	5.3	5.1
50	93.0	93.0	93.6	7.0	7.0	6.4

LApex: sample in low actinic glass; syringe covered; plate and tank exposed to light.
LAlight: sample in low actinic glass; syringe exposed to light; plate and tank exposed to light.
CGlight: sample, syringe, tank and plate exposed to light.
* For chromatographic conditions see text.

spotting solution was protected from laboratory light (in low actinic glassware) while the spotting and development were completed under light conditions. The same trend as before can be observed. The isomerization and decomposition however, occur at a slower rate than under laboratory light conditions. The results of the complete dark experiment (Figure

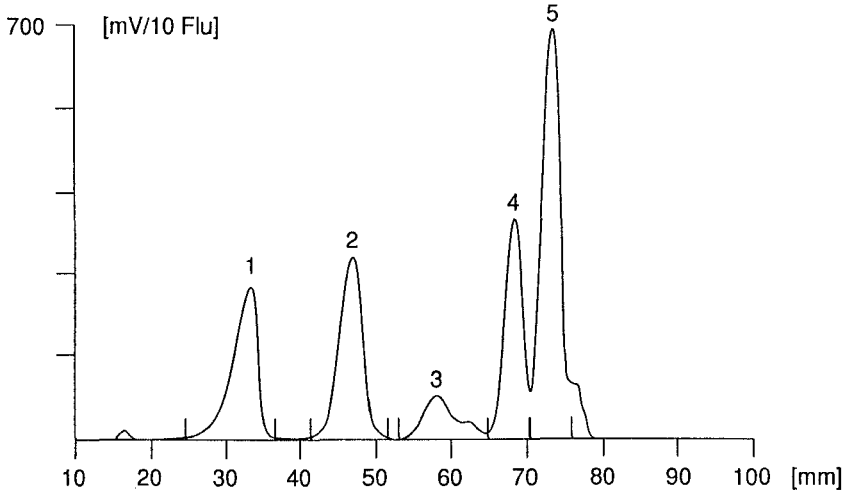


Figure 6. Chromatogram of a synthetic mixture of MK0679 and known impurities. 1 - MK0679 diacid; 2 - MK0679; 3 - MK0679 dimer; 4 - MK0679 diamide; 5 - MK0679 methyl ester. For chromatographic conditions see text.

5c) showed no extraneous spots or cis isomer occurring over one hour time interval.

The conclusion drawn from these experiments is that no cis isomer or other degradates are generated under dark conditions over the time required for the analysis. It was observed from data presented in Table 1 that if the sample preparation and the separation were completed in laboratory light, at least 3% of cis would be formed during the analysis.

Table 2

The R_f Values for MK0679 and Known Impurities

Compound	R_f* ± S.D.
Diacid	0.13 ± 0.01
MK0679	0.33 ± 0.01
Cis isomer	0.42 ± 0.01
Dimer	0.50 ± 0.01
Diamide	0.66 ± 0.01

* The R_f values represents the average of seven replicates. For chromatographic conditions see text.

Similar experiments and two dimensional TLC using the same mobile phase were completed under the dark conditions for the known impurities. No degradation was observed for the main compound or known impurities.

With the conditions described above, the quantitation of some known impurities was pursued. A chromatogram of a synthetic mixture of MK0679 along

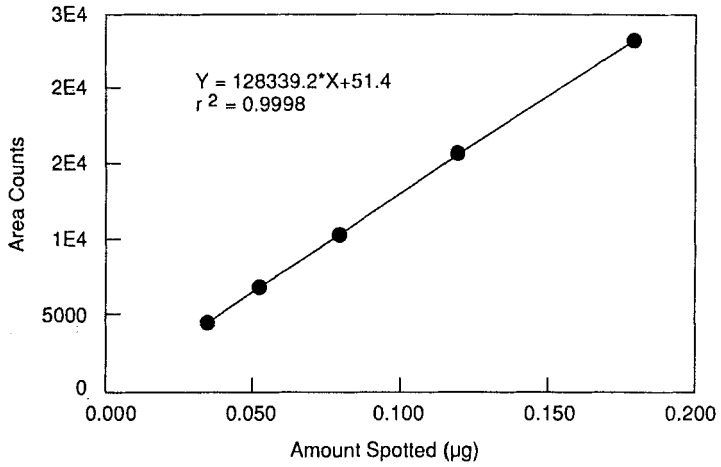


Figure 7. Calibration curve for MK0679 diacid. For chromatographic conditions see text.

with known potential impurities is presented in Figure 6.

The R_f of each component of the mixture is presented in Table 2. Solutions of increasing concentration were prepared, spotted and developed for quantitation.

The calibration curves were obtained for each impurity. They are presented in Figures 7, 8, 9, and 10. The correlation coefficients obtained were all $\geq$0.9998.

This suggests that the working concentration range of the method is in agreement with Beer's Law. A detection limit of less then 5 ng was obtained. The

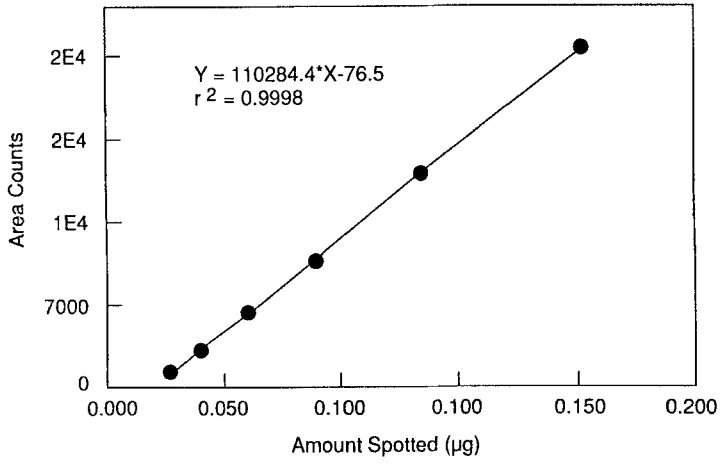


Figure 8. Calibration curve for MK0679. For chromatographic conditions see text.

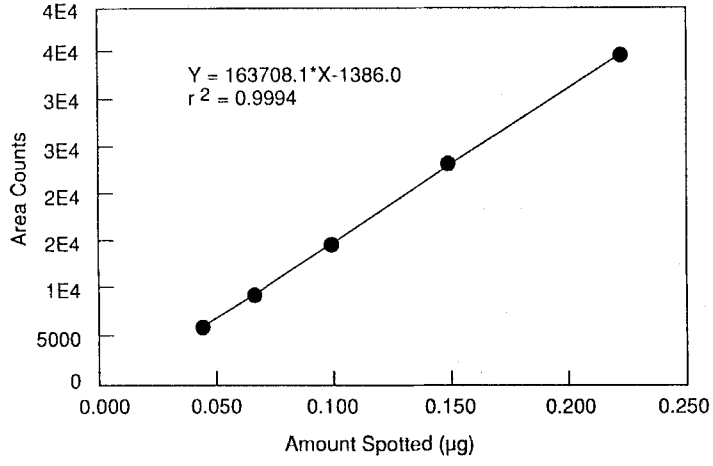


Figure 9. Calibration curve for MK0679 dimer. For chromatographic conditions see text.

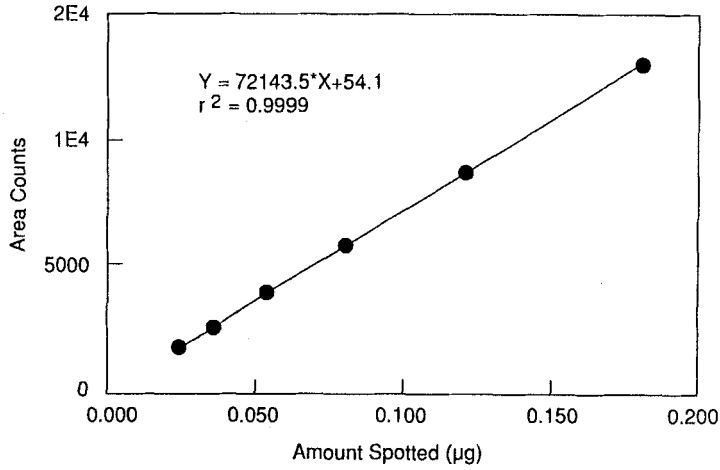


Figure 10. Calibration curve for MK679 diamide. For chromatographic conditions see text.

response factors (K_f) for each known impurity can be calculated as:

$$K_f = (\text{Area counts})/[\text{Amount spotted } (\mu g)]$$

Since all calculated response factors are within 10% and the amount expected of each impurity should be less than 0.5% (relative to the main component), an overall average response factor (K_f^*) was calculated to be 120,751 (cts/μg). In this case, the use of an average response factor does not affect the precision of the results.

Table 3

Recovery Experiments for MK0679 and Known Impurities*

Compound	Amount Recovered (μg)	
	Predicted	Found
Diacid	0.19	0.17
MK0679	0.18	0.19
Dimer	0.20	0.17
Diamide	0.20	0.16

* For chromatographic conditions see text.

In order to calculate the recovery of each impurity during the chromatographic separation, a synthetic mixture was prepared containing all known impurities each at a concentration of 0.05 mg/ml. To the plate was spotted 4 μl of this solution. The plate was developed and the recovery for each impurity can be calculated as follows:

Amount of impurity per spot (μg) = (Area counts)/K_f*

The recovery data are presented in Table 3.

From the experimental data, the observed response factor of the dimer is approximately 50% greater than those of the other components ($K_{fD} = 1.5K_f^*$). Therefore, the amount of dimer recovered can be calculated as:

$$\text{Amount dimer per spot } (\mu g) = (\text{Area Counts})/(1.5K_f^*)$$

In order to check the method, the weight percent analyses for two samples were completed. The sample solutions were prepared at a concentration of 0.5 mg/ml and 4 μl of this solution were spotted on the plate. The weight percent (Wt%) for each impurity was calculated as follows:

$$Wt\% = (A_{cs} \times 100)/(K_f^* \times W_s)$$

In this equation A_{cs} is area counts of the impurity peak and W_s is total amount sample spotted in μg. Two impurities were identified as diacid and diamide at a level of 0.2% and 0.1% respectively. One sample solution was spiked to achieve a final concentration of 1.5 μg/ml for each impurity. A 4 μl spot of this solution was developed and the recovery was

Table 4

Comparison of the Results from HPLC and TLC for Some Known Impurities

Compound	Wt% TLC*	Gradient HPLC
Sample 1		
Diacid	0.20	0.20
Diamide	0.10	0.08
Sample 2		
Diacid	0.20	0.20
Diamide	0.10	0.08

* For chromatographic conditions see text.

calculated. Subtracting the known amount added from the amount found, good recovery was obtained (data not shown). In order to further confirm the TLC method, two samples were analyzed by RP-8 gradient HPLC and the results for the known impurities were compared with those obtained by TLC. The results are presented

in Table 4. A good correlation can be observed between the two methods.

CONCLUSION

The results presented outline a quantitative method for the known impurities which may be found in MK0679.

In our experiments, we demonstrated that MK0679 undergoes cis/trans isomerization along with on-plate degradation. Our study suggests that under dark conditions no extraneous spots are generated over the time of analysis. In addition, under complete dark conditions, the compound and potential MK0679 impurities are preserved.

The recovery experiments indicate that the quantitation is rugged and can be used with confidence. A detection limit of less then 5 ng can be obtained. The calibration curves for each component are linear in the concentration range of analysis.

Acknowledgments

The authors wish to thank Ms. Rosemary Sblendorio and Dr. Dama Gopal for their valuable input towards the completion of this work.

REFERENCES

1. Gould, M. and Enzweiler, T., Planar Chromatography in the Life Science (J. C. Touchstone, ed.), Vol. 108, John Wiley & Sons, New York, 1990, p. 15.

2. Gocan, S., Modern TLC (N. Grinberg, ed.) Marcel Dekker, Inc., New York, in press.

3. Grassini-Strazza, G., Carunchio, V. and Girelli, A. M., J. Chromatogr., 466, 1, 1989.

4. Rama, N. V. and Tandon, S. N., J. Chromatogr. Sci., 16, 389, 1978.

5. Srivastava, S. P., Dua, V. K., Mehrotra R. N. and Saxena, R. C., J. Chromatogr., 176, 145, 1979.

6. Grinberg, N. and Weinstein, S., J. Chromatogr., 303, 251, 1984.

7. Jain, R. and Agarwal, D. D., J. Liq. Chromatogr., 6, 2661, 1981.

8. Chawla, H. M., Gambhir, I. and Kathuria, L., J. Chromatogr., 188, 289, 1980.

9. Lepri, L., Desideri, P. G. and D. Heimler, J. Chromatogr., 153, 77, 1978.

10. Lewis, S. and Wilson, I. D., J. Chromatogr., 312, 133, 1984.

11. Qureshi, M. and Sharma, S. D., Chromatographia, 11, 153, 1978.

12. Jain, A. K., Agrawal, S. and R. P. Singh, J. Liq. Chromattogr., 4, 2073, 1981.

13. Grinberg, N. Baiano, J. A., Bicker, G., Tway, P. and Ellison, D., Planar Chromatography in the Life Sciences (J. C. Touchstone ed.), Vol. 108, John Wiley & Sons, New York, 1990, p. 69.

14a. Young, R.N., Guindon, Y., Jones, T.R., Ford-Hutchinson, A.W., Belanger, P., Champion, E., Charette, L., DeHaven, R.N., Denis, D., Fortin, R., Frenette, R., Gauthier, J.Y., Gillard, J.W., Kakushima, M., Letts, L.G., Masson, P., Maycock, A., McFarlane, C., Piechuta, H., Pong, S.S., Rosenthal, A., Williams, H., Zamboni, R., Yoakim, C., Rokach, J., J. Adv. Prostaglandin, Thromboxane, Leukotriene Res., 16, 37, 1986.

14b. Hughes, D.L., Bergan, J.J., Amato, J.S., Reider, P.J., Grabowski, E.J.J., J. Org. Chem., 54, 1787, 1989.

14c. McNamara, J.M., Leazer, J.L., Bhupathy, M., Amato, J.S., Reamer, R.A., Reider, P.J., Grabowski, E.J.J., J. Org. Chem., 54, 3718, 1989.

15. McNamara, J. M., private communication.

16. Grinberg, N., Bicker, G., Tway, P. and Baiano, J. A., J. Liq. Chromatogr., 15, 3181, 1988.