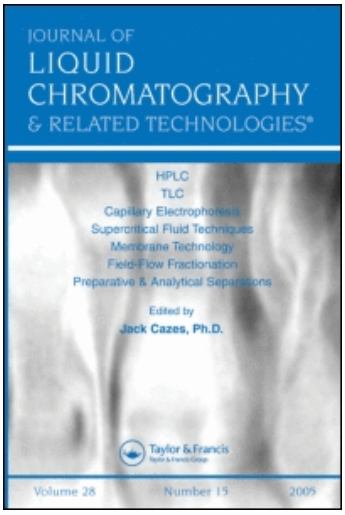

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SIMPLEX OPTIMIZATION OF DENSITOMETER PARAMETERS FOR MAXIMUM PRECISION IN QUANTITATIVE THIN LAYER CHROMATOGRAPHY

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ABSTRACT

Simplex optimization was employed for the selection of densitometer slit width and wavelength settings to maximize the reproducibility of the determination of diphenhydramine hydrochloride and pseudoephedrine hydrochloride in Benadryl-D™ capsules by thin layer chromatography. The two densitometer parameters were simultaneously adjusted in a systematic manner to minimize the sum of the squares of the relative standard deviations of the respective peak areas from the two active compounds on a developed thin layer chromatographic plate. The optimization process was followed graphically and resulted in the rapid establishment of a single set of densitometer parameters giving peak areas with relative standard deviations of less than 1% for each of the active components in the formulation.

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INTRODUCTION

The use of thin layer chromatography (TLC) as a quantitative technique has increased rapidly over the last several years, owing to improvements in the quality of commercial plates, the development of new sample application techniques, and the availability of scanning densitomers for the spectrophotometric analysis of developed plates. In the areas of pharmaceutical research and quality control, quantitative TLC has been successfully used for the assay of active components in a number of different types of formulations (1-3).

An important aspect of any chromatographic determination is the reproducibility of its results. In TLC analysis, this is governed by a number of factors, including sample preparation, plate homogeneity and the stability and reproducibility of the densitometric signal. The error associated with the densitometer can generally be minimized by careful adjustment of operating parameters. Although this is often accomplished by a trial and error method, studies have been made on the effects of some densitometer settings on the response from scanned TLC spots (4,5).

As part of an investigation of the factors which contribute to the indeterminate error in quantitative TLC, we have employed the technique of simplex optimization (6,7) for the adjustment of two densitometer parameters, wavelength and slit width, to achieve maximum precision in the quantitative TLC determination of diphenhydramine hydrochloride and pseudoephedrine hydrochloride in decongestant Benadryl-D™ capsules. The details of the optimization are described in this report.

EXPERIMENTAL

Materials: All chromatographic solvents used were HPLC grade (E.M. Science, Cherry Hill, NJ). Benadryl-D™ capsules were obtained from the Parke-Davis Pharmaceutical Research Division of Warner-Lambert Co. (Ann Arbor, MI). The capsule formulation consisted of 50 mg of diphenhydramine hydrochloride, 60 mg of pseudoephedrine hydrochloride, 130 mg of inert excipient material. Diphenhydramine hydrochloride was obtained from the Parke-Davis Research Division of Warner-Lambert Co. (Holland, MI). Pseudoephedrine hydrochloride was obtained from Ganes Chemical, Inc. (Carlstadt, NJ).

Apparatus: High Performance TLC plates (Silica Gel 60 10 x 10 cm, 0.2 mm layer thickness) were obtained from E.M. Science (Cherry Hill, NJ). Sample solutions were applied to the plates in 10 mm x 1 mm bands with a Camag Linomat III unit, using spray settings of 4.0 seconds/microliter and 50 mm/microliter. The developing solvent in all experiments consisted of 5 parts 2-propanol, 4 parts methanol, 0.5 parts concentrated ammonium hydroxide, and 0.25 parts water, by volume. Developed plates were scanned with a Camag Model 1 single beam densitometer, using a deuterium ultraviolet source, a scan speed of 0.5 mm/sec, a slit height of 0.6 mm, a span setting of 5.0 and a sensitivity setting of 12. Peak areas were measured using a Hewlett-Packard Model 3390A integrator. Ultraviolet spectra of methanol solutions of the active components of the formulation were obtained from 200-270 nm using a Perkin-Elmer Lambda 5 spectrophotometer. The wavelengths of maximum absorption were found to be 206 nm for pseudoephedrine hydrochloride and 204 nm for diphenhydramine hydrochloride.

Procedure: Each Benadryl-D™ capsule was prepared for analysis by dissolving its contents in 100 ml of the developing solvent. Five microliter samples of this solution were applied to a TLC plate, and the plate was developed in a linear chamber. For the determination of peak area relative standard deviations (R.S.D.'s), a single lane of the developed plate was scanned at least ten times under each of the slit width and wavelength settings specified in Table 1.

DISCUSSION

Since Benadryl-D™ capsules contain two active ingredients, the first requirement for their assay by TLC was that the chromatographic system should quantitatively separate the two compounds from each other and the inert materials in the formulation. The densitogram (Figure 1) of a developed plate from the TLC of a sample of a Benadryl-D™ capsule under the chromatographic conditions described earlier demonstrates that this requirement was met. The chromatographic system gave excellent separation of the two active ingredients, and no apparent interference by excipient materials in the formulation was observed.

Although quantitative information was required for both active ingredients in the formulation, a single set of densitometer parameters for the assay of both components was desirable, since this would limit the need for repeated scans of the developed plate once the method was put into routine use. Optimization of precision was complicated by this requirement, however, since the best densitometer conditions for the assay of one of the components could in theory be the worst conditions for the assay of the other.

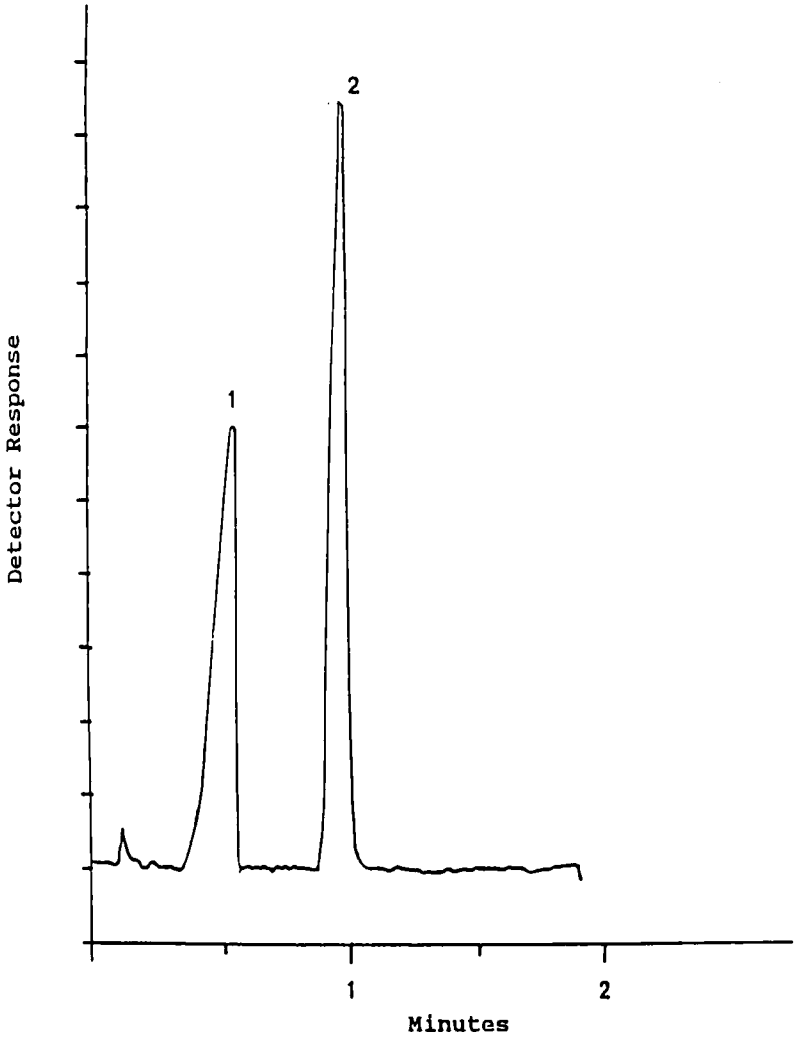

FIGURE 1. Densitogram of a developed plate from the TLC of a Benadryl-D™ capsule: wavelength = 215 nm, slit width = 2.0 mm. Components: 1 = diphenhydramine, 2 = pseudoephedrine.

For this reason, minimization of the individual relative standard deviations s_1 and s_2 peak areas from repeated scans of the two components on the developed plates was not performed. Alternatively, the goal of this optimization process was to minimize the sum S^2, of the variances (i.e., the squares of the R.S.D.'s) s_1^2 and s_2^2 of both peak areas from repeated scans: $S^2 = s_1^2 + s_2^2$. Like earlier work on the optimization of the sum of separation parameters between adjacent peaks in the gas and liquid chromatography of complex mixtures (8-10), this approach sought a set of instrumental parameters which did not optimize results for one component at the expense of the other.

Densitometer slit width and wavelength were chosen as the two parameters to optimize experimentally because they were judged as the most difficult to optimize on an intuitive basis. The wavelengths of maximum absorbance for both active ingredients in Benadryl-D™ were found to be between 200 nm and 210 nm, an area of the spectrum where formulation excipients and impurities on the TLC plate are also likely to absorb, and the power output of a deuterium light source is not at its highest. Because both of these factors could lead to variations in the densitometric responses for each component from a developed plate, it was not certain whether the minimum value for S^2 would be achieved in this wavelength range. Additionally, while it had been previously shown with standard compounds that signal-to-noise ratios increase with densitometer slit width (4), it was not clear how these earlier results would relate to densitometer peak area reproducibility in the TLC analysis of an actual formulated pharmaceutical sample. Neither was it known whether slit width is a variable that is completely independent of densitometer wavelength when considered in terms of peak area reproducibility. All of

these uncertainties made optimization of these two parameters by independently measuring their effects on S^2 difficult. As an alternative, the technique of simplex optimization was employed to arrive at the minimum value of S^2 by simultaneously varying both the wavelength and slit width parameters.

While multiparameter simplex optimization is often performed with the aid of computer algorithms (6, 7, 11), the optimization of only two parameters in this work allowed the procedure to be efficiently performed graphically. The simplex graph is shown in Figure 2, and the experimental process that was followed is as follows:

1. Three points were arbitrarily chosen on the graph of slit width vs wavelength, and values for S^2 were experimentally determined for the conditions specified at each of the points. These points make up triangle 1 (or simplex 1) in Figure 2, with the values of S^2 shown at each apex point.

2. Simplex 1 was graphically reflected away from the point of the least acceptable (i.e., the largest) value of S^2, establishing simplex 2. The value of S^2 for the conditions specified by the new point in this simplex was then determined.

3. Since reflection of simplex 2 away from the point with the largest S^2 value would have led back to simplex 1, a reflection was made away from the second least acceptable point, establishing simplex 3. Again, the value of S^2 was determined under the conditions of the newly-established point.

4. The processes described in steps 2 and 3 were repeated, giving rise to simplexes 4-9, for which values of S^2 under conditions of the appropriate points were determined.

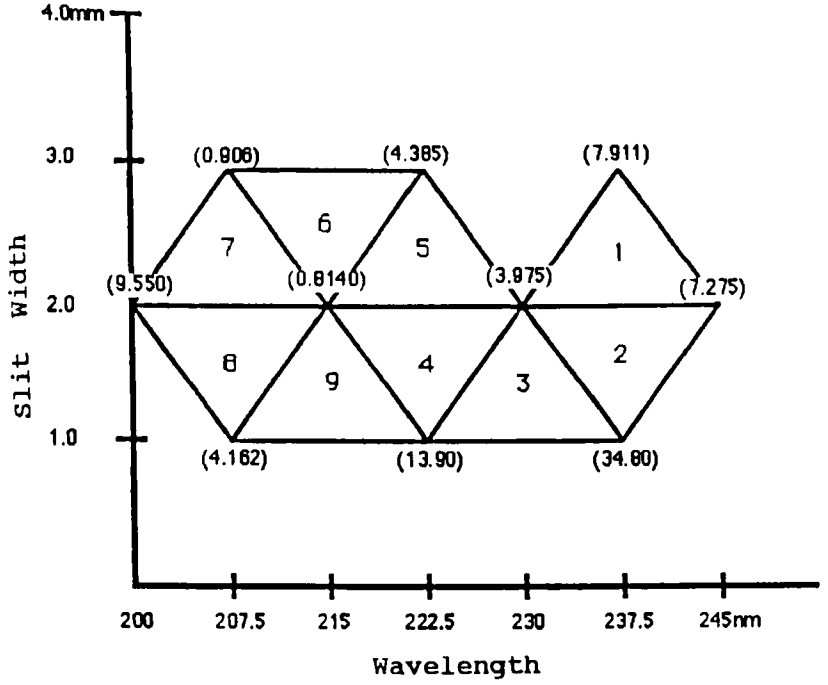

FIGURE 2. Simplex diagram for the optimization of densitometer slit width and wavelength. Parenthetical numbers refer to the value of S^2 at each point.

The optimization process was complete after acquiring data for simplex 9, since its reflection according to any of the above rules would have given rise to a previously established simplex. The optimum parameters established by the procedure correspond to the single common point in simplexes 4-9, which are a slit width of 2 mm and a wavelength of 215 nm.

The relative standard deviations of the individual peak areas for the two active components of the formulation under each of the conditions used in the optimization are listed in Table 1. The optimum slit width and wavelength settings

TABLE 1

Densitometer Settings and Corresponding Peak Area Relative Standard Deviations

Wavelength (nm)	Slit Width (mm)	s_1	s_2	S^2
245.0	2.0	1.089	2.468	7.275
237.5	3.0	0.490	2.770	7.911
220.0	2.0	0.506	1.928	3.975
237.5	1.0	0.964	5.820	34.80
222.5	1.0	0.295	3.716	13.90
*215.0	*2.0	*0.245	*0.868	*0.814
222.5	3.0	0.256	2.078	4.385
207.5	3.0	0.374	0.875	0.906
200.0	2.0	1.486	2.710	9.550
207.5	1.0	0.665	1.929	4.162

s_1 = R.S.D. of diphenhydramine peak area, s_2 = R.S.D. of pseudoephedrine peak area, $S^2 = s_1^2 + s_2^2$

* Parameters for minimized S^2.

established by the simplex process resulted in peak area R.S.D.'s of 0.245% for diphenhydramine hydrochloride and 0.868% for pseudoephedrine hydrochloride. Further refining of parameters is theoretically possible by investigating S^2 values in smaller-sized simplexes near the optimized point (6, 7), but in this application it was judged to be unnecessary since the peak area variabilities for both compounds were already at acceptable levels (12).

Although the simplex procedure does not establish a cause and effect relationship between optimized parameters in any application, it is interesting in this study that the optimized wavelength determined by the process was not the wavelength of maximum absorbance for either of the

compounds, nor was the optimized slit width at its highest possible setting. This could be the result of the interference and/or source power effects on densitometer peak area discussed earlier.

CONCLUSIONS

In this work, application of the simplex optimization method has allowed the rapid determination of densitometer slit width and wavelength settings for maximum peak area reproducibility in the TLC assay of a formulated capsule containing two active ingredients. Minimizing the sum of the peak area variances allowed the determination of a single set of optimum parameters which gave acceptable results for the quantitative analysis of both components.

In a TLC assay such as this one, the indeterminate error associated with the densitometer is only one component of the total variability in the analytical results. Other sources of error included those related to sample preparation and application to the plate, and the homogeneity of the plate itself. We are currently investigating the relative magnitudes of these sources of error and methods for their minimization. Results will be reported in a later report.

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