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SHORT COLUMN CHROMATOGRAPHY FOR A ROBOTIC DISSOLUTION ASSAY OF ADINAZOLAM MESYLATE TABLETS

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ABSTRACT

The use of robotics can potentially increase the productivity of pharmaceutical quality control laboratories, especially in areas such as dissolution testing. However, if the speed of the assay is limited by factors other than the speed of the robot, such as chromatography, the productivity of the assay is less than optimal. A robotic dissolution assay for adinazolam mesylate tablets is currently under development. One of the obstacles to implementation is that the productivity of the assay is limited by a chromatographic analysis time of six minutes per sample. This report descibes the application of short column chromatography to reduce the analysis for dissolved drug to under two minutes. Three short columns were evaluated for use in dissolution rate assay of adinazolam mesylate tablets. In this case, the application of short column technology allows for a potential increase in productivity of about 75 percent. Similar productivity gains may be achieved for other robotic assays that are limited by the speed of the chromatography.

INTRODUCTION

The use of robotics can potentially increase the productivity of pharmaceutical quality control laboratories,

especially in areas such as dissolution testing. However, in order to realize substantial productivity gains, it is necessary to have the total assay time for a lot limited by the speed of the robot and not by other factors such as chromatography. A robotic procedure is currently being developed by the Control Division of The Upjohn Company to assay adinazolam mesylate tablets for dissolution rate. The Zymate II robot (Zymark Corp., Hopkinton, Mass.) employs asynchronous timing so that while the robot is performing a number of functions, a new dissolution sample is ready for the chromatograph every six minutes. The retention time for adinazolam mesylate with the current chromatographic system is about five minutes, consequently, the total time needed to assay a batch of six tablets with two test points (15 and 30 minutes) is about 140 minutes. If the analysis for dissolved drug can be performed in less than two minutes, the rate of the determination is no longer limited by the analysis time and the robot can be operated in a batch mode (i.e. perform the same function on all six dissolution flasks before moving on to the next batch). In this mode the dissolution experiment for one lot of tablets would take about 80 minutes, thereby increasing the potential assay throughput by about 75 percent.

Short column chromatography was chosen as a means to obtain short analysis times. This technology uses analytical columns that are usually between 3 cm and 5 cm in length and are packed with small particles, typically three to five microns in diameter. As the diameter of the packing particles decreases, column efficiency increases. The application of short column chromatography to achieve fast analysis times for dissolution samples has been discussed previously (1-3). This discussion describes an application of short column technology for use in a robotic dissolution assay of adinazolam mesylate tablets that will

reduce the analysis time to under two minutes without sacrificing the quality of the chromatogram.

MATERIALS AND METHODS

Adinazolam mesylate tablets (The Upjohn Company, Kalamazoo, MI) were dissolved in 500 ml of 0.05 M pH 7.0 phosphate buffer at 37.0 ^{o}C +/- 0.5 ^{o}C with a Vanderkamp 600 Six Spindle Dissolution Tester (Van-Kel Industries, Chatham, NJ) using USP method II (rotating paddles) at 50 rpm. The dissolution samples were withdrawn manually using a syringe and filtered through a 5.0 micron disposable filter (part #4199, Gelman Sciences, Ann Arbor, MI). The dissolution samples were analyzed on a reverse-phase chromatographic system using a mobile phase of acetonitrile (American Burdick & Jackson, Muskegon, MI): tetrahydrofuran (American Burdick & Jackson, Muskegon, MI): 0.05 M pH 7.0 phosphate buffer in the ratio of 35:5:60 at a flow rate of 1.5 milliliters per minute using a dual piston pump (model # 100A, Altex Scientific Inc., Berkeley, CA). Injections were made with an Upjohn Autosampler (The Upjohn Company, Kalamazoo, MI) using either a 100 microliter or 10 microliter sample loop. The chromatographic columns used in this investigation were a Brownlee RP-8, Spheri-10, 13cm (10 cm analytical cartridge + 3 cm guard catridge) x 4.6 mm column (the column currently used in the assay) (I); a Brownlee RP-8, Spheri-10, 3 cm x 4.6 mm guard column (II); a Brownlee Velosep RP-8, 3 micron, 4 cm x 3.2 mm short analytical column (III); and a Perkin-Elmer C-8, 3x3, 3 cm x 4.6 mm short analytical column (IV). Detection was by ultraviolet absorption using a variable wavelength detector (Spectromonitor D, Laboratory Data Control, Riviera Beach, FL) at 254 nm. The durability of each of the short columns was evaluated by making continuous injections onto the columns and monitoring changes in peak shape, such as peak splitting and/or tailing and separation from the solvent front.

RESULTS AND DISCUSSION

The purpose of this investigation was to evaluate different short chromatographic columns and not the Zymark robotic system. Therefore, adinazolam mesylate tablets were dissolved, manually withdrawn and filtered, and transferred to a liquid chromatograph. They were analyzed for dissolved drug using the four chromatographic columns listed above. Each of the three short columns (II-IV) gave mean results that were not statistically different from the accepted values, obtained using column I. Table 1 shows some comparative dissolution results for one lot of adinazolam mesylate tablets chromatographed on the conventional length column and the three short columns. All of the chromatographic parameters were kept constant with the exception of the injection volume. In the case of those columns packed with three micron particles (columns III and IV), the decreased retention volume and shorter column length result in less sample dilution. In addition, due to increased efficiency, the band widths become narrower. The combination of these effects results in a substantial increase in peak height. In order to keep the peak on scale, it was necessary to reduce the injection volume from 100 microliters to 10 microliters. Figure 1 shows chromatograms obtained from each of the respective columns.

Although all of the short columns studied gave results that were not statistically different from the accepted values, the durability of the short columns was somewhat different. Column II was the most durable and was still producing acceptable chromatograms after over 1000 injections. Columns III and IV began to show peak splitting after about 460 and 770 injections, respectively. Also, because columns III and IV require a 10 microliter injection, some minor modifications to the robot-chromatograph interface would be required. Taking durability and ease of transition into consideration, column II, the Brownlee RP-

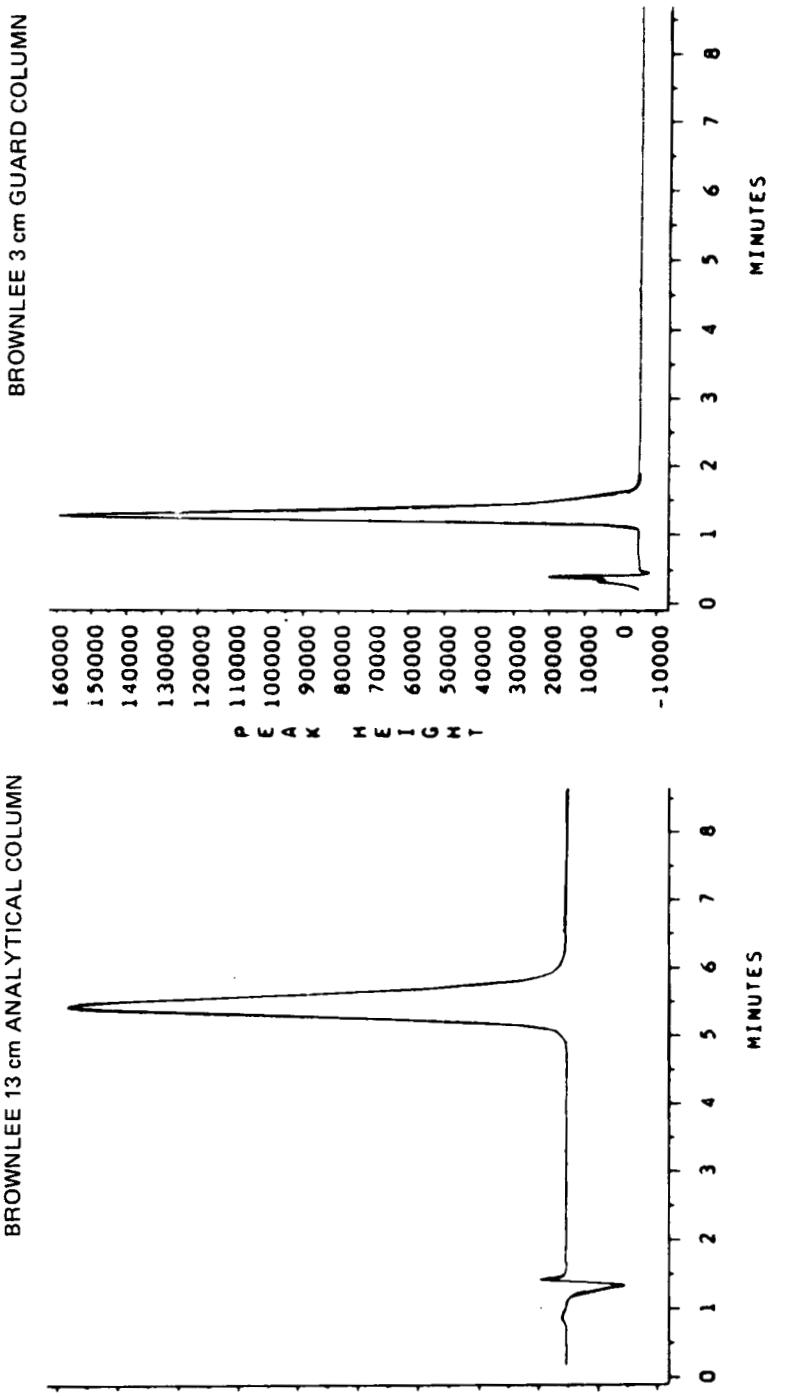
TABLE 1

Comparative Dissolution Results for Six Individual Tablets from One Lot of Adinazolam Mesylate Tablets

Test Point (minutes)	Percent Dissolved			
	[a] I	[b] II	[c] III	[d] IV
15	96	96	101	98
	79	80	79	80
	84	85	89	88
	82	82	84	85
	90	90	93	95
	97	97	96	101
MEAN	88	88	90	91
RSD (%)	8.5	8.1	8.9	8.9
T (obs) [e]	---	0	0.44	0.66
30	99	98	105	97
	86	86	88	84
	91	91	92	91
	89	87	93	88
	94	94	91	94
	99	99	96	100
MEAN	93	92	94	92
RSD (%)	5.7	5.9	6.3	6.4
T (obs) [e]	---	0.32	0.31	0.31

[a] Brownlee RP-8, Spheri-10, 13 cm x 4.6 mm, analytical column.
[b] Brownlee RP-8, Spheri-10, 3 cm x 4.6 mm, guard column.
[c] Brownlee Velosep RP-8, 3 micron, 4 cm x 3.2 mm short column.
[d] Perkin-Elmer C-8, 3x3, 3 micron, 3 cm x 4.6 mm short column.
[e] T (alpha/2) = 2.228 for ten degrees of freedom at the 95 percent confidence level.

8, Spheri-10, 3 cm x 4.6 mm guard column, was determined to be the best choice for the robotic dissolution assay of adinazolam mesylate tablets. Inter-lot column reproducibility of the chromatography from guard column to guard column was verified by testing different lot numbers of column II (see Table 2). There



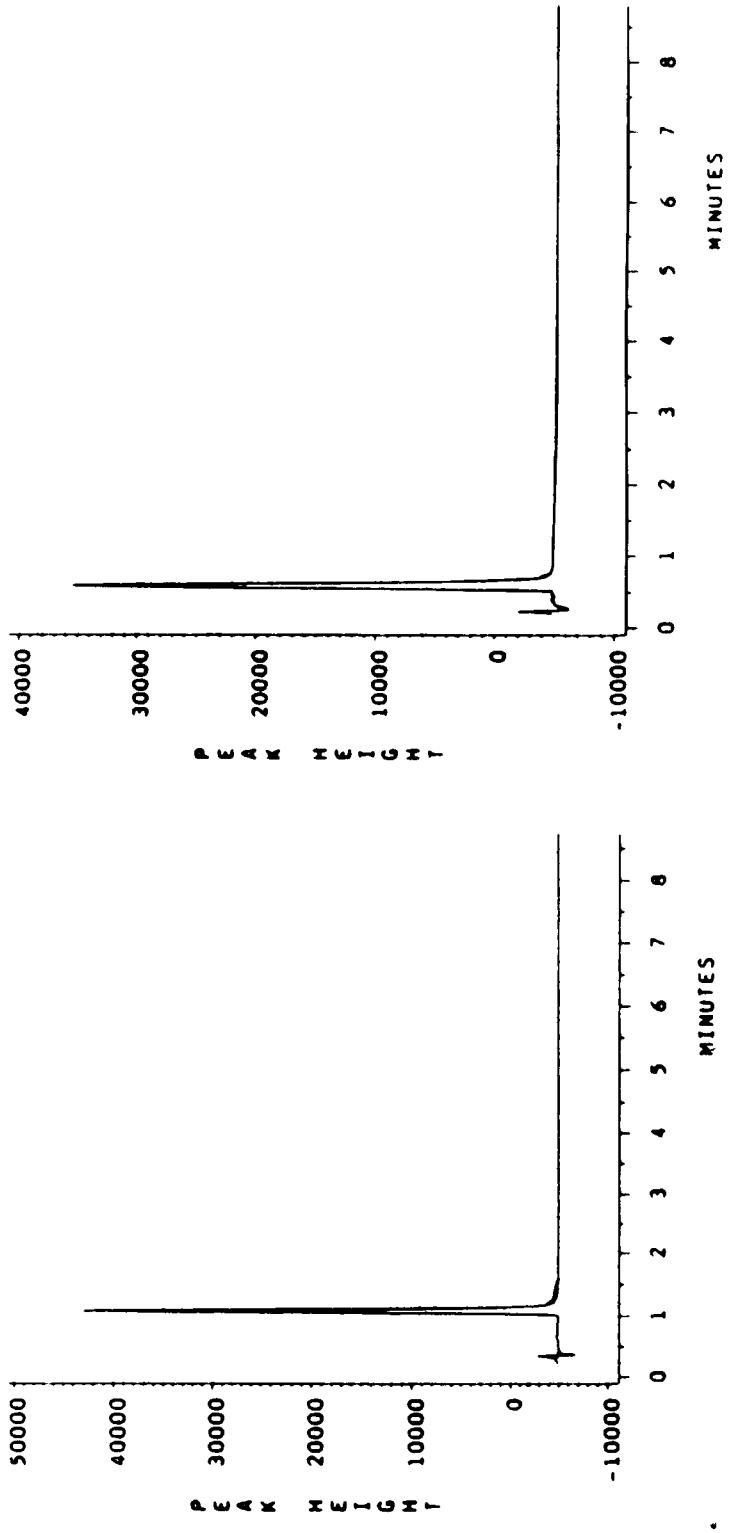


FIGURE 1.
Chromatograms of adinazolam mesylate obtained from the conventional length column and the three short columns.

TABLE 2

Comparison of the Performance of Three Different Brownlee RP-8, Spheri-10 3 cm x 4.6 mm Guard Columns

Parameter	Column "A"	Column "B"	Column "C"
Lot Number	unknown	01157A	04157B
Retention (minutes)	1.03	1.01	1.03
Symmetry	148	171	156
Theoretical Plates	337	287	308
Tail Factor	1.43	1.56	1.4

were no significant differences in factors such as retention time and theoretical plates between different lots of column II. Replacing the conventional length column with the 3 cm guard column reduces the chromatographic analysis time by five minutes per sample and can potentially increase the throughput of the assay by about 75 percent.

Due to the increased response and column efficiency achieved with three micron particle packings, column IV, the Perkin-Elmer C-8, 3x3, 3 cm x 4.6 mm short column, may be applicable in cases where changes in the formulation of adinazolam mesylate tablets require improved response or resolution. Column III, the Brownlee Velosep RP-8, 3 micron, 4 cm x 3.2 mm short column would not be suitable for routine use for the dissolution assay of adinazolam mesylate tablets due to its relatively poor durability.

CONCLUSION

Short column technology has been demonstrated to be a viable means of reducing sample analysis time and therefore increasing the productivity of a robotic assay. In the case of the

dissolution assay of adinazolam mesylate tablets, short column chromatography allows for faster analysis of dissolved drug and a potential increase in assay productivity of about 75 percent. Short column technology may be useful in increasing the productivity of other robotic dissolution assays that are currently limited by the speed of the chromatography.

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