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Short Communication

Utility of column switching to analyse low dose dissolution samples for pharmaceutical formulations

C. BELL* and D.P. IP

Department of Pharmaceutical Research and Development, WP26-331, Merck Research Laboratories, West Point, PA 19486, USA

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Introduction

An automated HPLC method which uses column switching to concentrate and analyse dissolution samples from a low dose pharmaceutical formulation is described. Traditionally, column switching has had many applications, including clean-up of biological samples [1–12] and trace enrichment of environmental samples [13–16]. Recently, the technique has gained popularity in multidimensional chromatography [17–18] as well as pharmaceutical analysis [18–22]. Dissolution testing of pharmaceutical formulations is normally performed in 900 ml of media [23]. It is therefore obvious that the analytical sensitivity for the determination of dissolution samples, particularly at very low concentrations, is very important. Of similar concern would be dissolution testing of formulations containing weak UV-absorbing drug candidates. By using column switching, dilute samples from dissolution testing can be concentrated by making several injections onto a guard column, where the drug is retained, and then eluting the drug onto the analytical column. The column switching technique gives better precision than the traditional direct injection method for very dilute samples. Column switching for dissolution samples has been done previously in the back-flushing mode [18, 24]. As reported in these papers, the active ingredient from dissolution samples is concentrated on a pre-column, and back-flushed into the main analytical column(s) for separation. The back-flushing technique is essential to remove interference from formulation constituents. The reported relative standard deviation of these determinations was less than 2%.

Tablet formulations of L-657,743-002W which contain 50 μg, 100 μg, or 1 mg of drug per 100 mg tablet have been manufactured. This paper deals only with the 50 μg tablet. L-657,743-002W was an experimental drug for evaluation as an antidepressant [25]. This paper will describe an HPLC method which uses column switching to concentrate the dilute sample expected from the dissolution assay of the 50 μg tablets. Precision data obtained by column switching and direct injection methods are compared.

Experimental

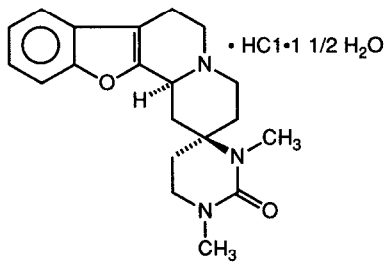
Materials

L-657,743-002W (Fig. 1) bulk drug was prepared at Merck Research Laboratories (Rahway, NJ, USA). Tablet formulations of L-657,743-002W and the placebo were provided by Merck Research Laboratories (West Point, PA, USA).

Routine dissolution

The routine dissolution experiments were performed in an adjacent laboratory. The dissolution was carried out in 500 ml of 0.22 M sodium acetate buffer, pH 4.5, at 37°C, using

* Author to whom correspondence should be addressed.



L-657,743-002W

Figure 1
Molecular structure of L-657,743-002W.

USP paddles at 50 rpm. A 500 ml volume was used instead of the 900 ml previously mentioned in order to increase the sensitivity for this low potency sample. A 5 ml volume of sample was removed after 60 min and assayed versus a standard of approximately the sample concentration (0.12 μg ml^{-1}) by HPLC. The routine LC assay employed a Waters μBondapak ODS column, 30 × 0.39 cm, with a mobile phase of phosphoric acid, pH 3.0, containing 0.1% sodium hexane sulphonate–acetonitrile (76:24, v/v). The specified μBondapak column could not be used for the column switching experiments since it would not fit in the HP1090 oven with the switching valve installed. The oven temperature was 40°C and the detection wavelength was 245 nm. Results of this experiment are reported in Table 1.

Table 1
HPLC data for duplicate injections of 60 min dissolution samples

	Formulation A		Formulation B	
Sample	Area	% Difference*	Area	% Difference*
	4343		2803	
1	4470	2.9	2961	5.5
	3546		3354	
2	2849	22	3252	3.1
	3431		3439	
3	3608	5.0	3265	5.2
	3307		3298	
4	3497	5.6	3029	8.5
	3749		3050	
5	3579	4.6	3352	9.4
	3404		3261	
6	3620	6.2	3289	0.9
	$\bar{X}$ =	8.2	$\bar{X}$ =	5.4

*Between areas of replicate injections.

Instrument and LC conditions

For the present experiments, the analyses were performed on an HP1090A HPLC (Hewlett–Packard, Avondale, PA, USA) equipped with an HP1040 diode array detector and a six-port column switching valve (Hewlett–Packard, Part No. 79826A). The integrator used was an HP3392A. All were controlled by an HP85B microprocessor. A phenyl analytical column (150 × 4.6 mm i.d., manufactured by either IBM or Jones) was used with a mobile phase of water–acetonitrile (HPLC grade, Fisher Scientific)–0.05 M potassium phosphate monobasic (HPLC grade, Fisher Scientific), pH 3.0 (30:45:25, v/v/v). Column temperature was 35°C and the UV detection wavelength was 210 nm.

Column switching scheme

Figure 2 is a sketch of the switching valve. A Brownlee RP-8 guard column (30 × 4.6 mm i.d.) was inserted in the column inlet line before the switching valve. The two possible routes through the switching valve were SW = 0 and SW = 1. In the SW = 0 position (solid line), the analytical mobile phase was pumped through the guard, the Phenyl column, the detector and then to waste. In the SW = 1 position (dashed line), HPLC grade water was pumped through the guard column, a shunt,

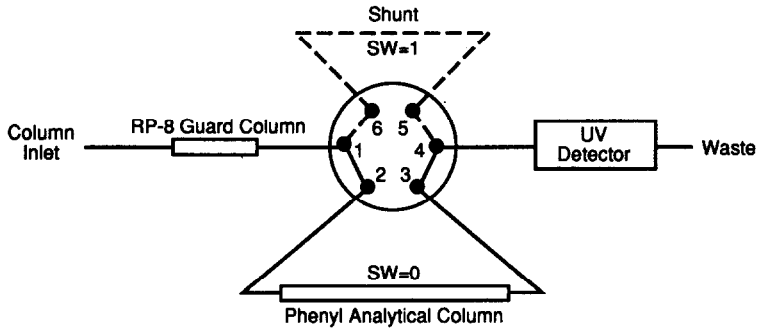


Figure 2
Sketch of the flow pattern through the column switching valve.

the detector, and then to waste. In both positions the flow rate was 1.4 ml min^{-1}. In the SW = 0 position, there was no flow through the shunt and, in the SW = 1 position, there was no flow on the analytical column.

Column switching operating sequence

The operating sequence of the column switching technique is outlined in Table 2. The analytical column was first equilibrated at 35°C with mobile phase (SW = 0, Step 1) prior to the start of the assay. To start the assay, the valve was switched to SW = 1 (Step 2), and the guard column was equilibrated at 35°C with 100% water for 1 min prior to injection. Four successive 250 μl injections were then made onto the guard column (Step 3), with the run time set at 30 s. (At least 30 s was required for the plunger to return to its original position in the syringe.) The drug was retained at the top of the guard column, while the sample solvent was eluted to waste.

After the fourth injection, the instrument automatically switched the valve to the SW = 0 position (Step 4). The mobile phase eluted the drug as a single peak through the guard column, the analytical column and then to the detector (λ = 210 nm). Since no injection was made when the switch occurred, the integrator started immediately. The retention time for the drug was about 5.8 min. This whole operation took less than 20 min. Detection wavelength (210 nm) was optimized to increase sensitivity, as compared to the wavelength described in the Routine dissolution section.

Selection of standard/sample diluent

A recovery experiment simulating the dissolution concentration was performed by column switching to aid in the selection of an appropriate solvent for the potency assay. The experiment was designed to compare recovery of the drug from placebo using different solvents. For the experiment, a stock solution was prepared to a concentration of 12 μg ml^{-1} in water. Three aliquots of stock solution were then diluted to a concentration of 0.12 μg ml^{-1} in three different solvents: water; 0.05 M phosphate; and water–acetonitrile (75:25, v/v), and used as standards. For the samples, 5.0 ml of the stock solution was added to a crushed placebo tablet and diluted to 500 ml (final concentration, 0.12 μg ml^{-1}) with the same three solvents, and measured against the appropriate standard.

Table 2
Operating sequence of the column switching technique

Step	Switching sequences of valve	Event
1	SW = 0	Phenyl analytical column is equilibrated with mobile phase prior to start.
2	SW = 1	Guard column is equilibrated with water for 1 min.
3	SW = 1	Four 250 μl injections are made onto the guard column.
4	SW = 0	Valve is switched to analytical column, pumps switch to mobile phase, and integrator starts. Drug is eluted as one peak by the mobile phase onto the phenyl column.
5	SW = 0	Procedure begins again with Step 2.

Results and Discussion

Routine dissolution

The HPLC data shown in Table 1 were obtained from the 60 min sampling of the dissolution assay for two separate experimental lots (Formulations A and B) of tablets tested. The reproducibility of duplicate injections was poor, as shown by the peak areas in Table 1. For 12 tablets, the individual differences in areas ranged from 0.9 to 22%. The average of the differences was 8.2% for Lot A and 5.4% for Lot B. Normally, a 2% difference between duplicate injections by HPLC would be considered acceptable. In order to improve the reproducibility of the assay, an increase in the sample concentration was necessary. For this it was decided to investigate column switching.

Column switching — chromatography

The objective of column switching was to pre-concentrate the dilute sample solution initially at the top of the guard column while the sample solvent eluted to waste. The pre-concentrated drug was subsequently switched to the analytical column with the analytical mobile phase.

Figure 3 shows a comparison of the chromatograms obtained by (a) direct injection of a standard solution (0.12 μg ml^{-1} in water) using the HPLC conditions for column switching, (b) column switching (4×) of the same standard solution, and (c) column switching of a placebo solution. The baseline was automatically re-zeroed in the chromatograms after the mobile phase change caused baseline perturbations and after the valve was switched. Chromato-

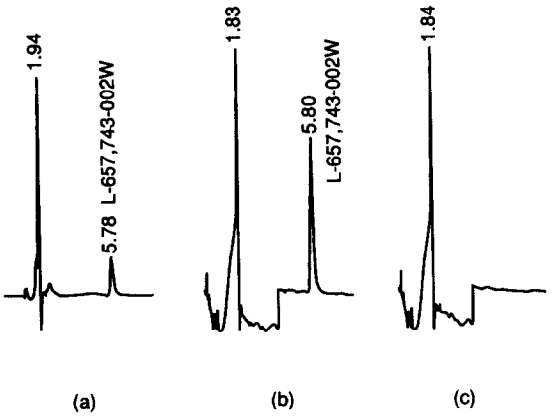


Figure 3
Chromatogram of (a) a direct injection of L-657,743-002W, (b) an injection of L-657,743-002W by column switching, and (c) an injection of a placebo by column switching.

gram (a), due to its small size, was subject to inconsistent integration which resulted in high RSDs of integrated peak areas, but in (b), the increased size of the peak provided more consistent integration. In (c), no interference from the placebo was observed.

Column switching data

The data in Table 3 show a comparison of 250 μl direct injections versus four 250 μl injections using column switching. The standards and samples were prepared in different solvents, as described in the Selection of standard/sample diluent section, at the dissolution concentration of 0.12 μg ml^{-1}. The solutions were analysed first using column switching and later on the same day by direct injection (using the HPLC conditions for column switching) in order to provide a comparison between the recovery data and the simulated dissolution data. As shown in Table 3, the means of the per cent difference (0.6, 1.0 and 0.4) for each solvent are clearly lower by column switching than the means (1.2, 6.2 and 4.1) obtained by direct injection, respectively. The column switching technique also reduced the difference in reproducibility between solvents. The individual per cent differences by

Table 3
Per cent difference between injections for direct injections (done in triplicate) vs column switching (done in duplicate)

		Direct injection (250 μl)			Column switching (4 × 250 μl)			
	Solvent	Area	% Difference	Mean % difference	Area	% Difference	Mean % difference*	% Recovery†
		2129			8449			
Std	A	2133	0.3			0.3		
		2136			8427			
		1830			7420			
Sample	A	1843	2.2	1.2		0.2	0.6	88
		1870			7407			
		2077			8398			
Std	A	2081	1.2			1.4		
		2102			8278			
		2202			8244			
Std	B	2123	6.1			0.5		
		2071			8202			
				6.2			1.0	
		2019			7632			
Sample	B	1981	6.3			1.4		93
		1895			7741			
		2145			8422			
Std	C	2133	2.8			0.5		
		2194			8466			
				4.1			0.4	
		2203			8449			100
Sample	C	2218	5.3			0.2		
		2322			8468			

Solvent A = water, B = 0.05 M phosphate, C = 25% acetonitrile in water.
*Within each solvent group.
†Calculated versus the respective standard.

column switching were well below the 2% limit for differences between duplicates. The data show that column switching can be a valuable and effective technique for improving quantitation in samples of low concentrations.

Recovery

As mentioned previously, the recovery experiment simulating the dissolution concentration was performed by column switching to select an appropriate solvent for the potency assay. Results of the recovery experiment showed that when samples were quantitated versus the standard prepared in the same solvent, water and phosphate gave inadequate absolute recovery (88 and 93%, respectively) of drug from placebo. Solvent C, 25% acetonitrile in water, showed complete recovery (100%) of drug from the placebo. Since water, which showed the best reproducibility by direct injection, could not be used as a diluent for the tablet preparation in the potency assay, column switching showed a distinct advantage. It provided more precise and accurate data for the three solvents studied and thus identified 25% acetonitrile as a suitable diluent for the potency assay.

Linearity

Linearity by column switching was performed using six solutions ranging from 50 to 200% of the target dissolution concentration of 0.1 $\mu g\ ml^{-1}$. The results indicate that the curve was linear within this range; thus the linearity is sufficient for accurate quantitation.

Conclusion

It can be concluded that the column switching HPLC analysis is applicable to dissolution samples of low concentration. The method gives more precise data than the direct HPLC analysis and can be a valuable alternative technique in routine analysis of pharmaceutical formulations when low assay concentration is involved.

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