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Automated sample preparation of Roxifiban tablets: transfer of a manual method to an automated workstation

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Abstract

Automation offers obvious advantages for the preparation of tablets prior to analysis by HPLC including unattended operation, minimization of human intervention and an electronic audit trail. However, significant effort has to be put in up front to develop and validate an automated method, particularly if it is required to closely follow an existing manual method. Here, method transfer for Roxifiban, a fibrinogen receptor antagonist, will be discussed. A Zymark tablet processing workstation II (TPWII) was used for all automated sample preparations. Manual methods for composite assay, content uniformity, weight variation and degradation products testing of a tablet formulation were transferred to the TPWII. The method involved weighing of the sample, disintegration of the dosage form by homogenization, extraction of the analyte in the homogenate solution, filtration of the homogenate, dilution of the filtrate and transfer to autosampler vials. Obstacles to a quick transfer included limitations in the volume capabilities of the TPWII, poor analyte solubility and achieving proper conditioning of the transfer lines and filter. After resolving these issues, a validated method was achieved. Spiked recoveries were from 99.4 to 101.1% (RSD's $< 0.5\%$). A cross-validation between automated and manual assay methods was compared by Westlake analysis giving a 0.7% calculated interval at the 95% confidence level. Carryover was 0.07% after 20 sample preparations at the highest tablet strength. © 2000 DuPont Pharmaceuticals Company.

Keywords: Automation; Roxifiban; Method development; Robotic sample preparation

1. Introduction

The routine task of sample preparation prior to HPLC analysis of pharmaceutical formulations is a prime example of a procedure that may well be efficiently automated. Just such an opportunity existed in the development of analytical methods for Roxifiban tablets. Although manual methods validated according to ICH guidelines already existed for this compound, the benefits of an automated method were expected to include continuous operation, increased precision, reduced solvent waste, an electronic audit trail and addi-

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tional free lab time and reduced chemical exposure for the analyst. However, in order to develop and validate an automated method, various concerns must be dealt with. Issues can exist such as limitations in the flexibility of the robotic workstation and the software and compatibility problems between the compound, solvents and internal surfaces of the instrument.

Roxifiban (Fig. 1), a fibrinogen receptor antagonist in phase IIB clinical studies [1,2], offered a good opportunity to utilize the benefits of an automated method. In this case, converting the existing manual method to an automated method did present some difficulties. Obstacles included selection of homogenization and dilution volumes to assure solubility of the active drug, the setting of proper rinse volumes to enable quantitative drug transfer and finding a balance in the clean-up procedures that gave acceptable carryover results with the minimum amount of solvent waste. After addressing all of the concerns in both the method development and validation processes, a validated automated sample preparation method was achieved that duplicated the results of the existing validated manual method.

2. Experimental

2.1. Manual method

2.1.1. Standard preparation

The standard preparation begins by accurately weighing approximately 230 mg of reference standard in a 200 ml volumetric flask then bringing to volume with dilution solvent (14:86, acetonitrile: 0.5% (v:v) glacial acetic acid in deionized water).

Roxifiban (DMP 754)

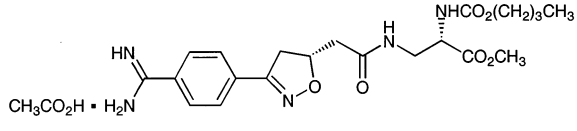

Fig. 1. Chemical structure of Roxifiban.

From this a 1:50 dilution in dilution solvent is made to prepare the assay standard and then a 1:100 dilution of the assay standard in dilution solvent results in the 1% degradation standard.

2.1.2. Sample preparation

The sample preparation for the composite assay and the degradation samples are the same, only the HPLC injection volumes differ. For composite assay, multiple tablets are placed in a volumetric flask to give a final concentration of 0.02 mg/ml for all tablet strengths except for the 1.5 mg tablets, in which case the final concentration is 0.0195 mg/ml. Each strength tablet has the same proportion of active drug relative to the total tablet weight. The flask is then approximately half filled with the previously mentioned dilution solvent. This is stoppered and allowed to shake on a mechanical shaker for 30 min after which the flask is filled to volume with dilution solvent and mixed. The final sample is then filtered through a 0.45 μm nylon 66 autovial syringeless filter. For content uniformity the same steps are followed only using one tablet per preparation in an appropriately smaller size volumetric flask. This is done for ten individual tablets per content uniformity study and each is individually weighed to obtain the weight variation data. These samples have been shown to be stable for up to 9 days.

2.2. Automated method

A Zymark Corporation (Hopkinton, MA) Tablet Processing Workstation II (TPWII) was used for all standard and sample preparations. This instrument consists of several functionally distinct components. There is a single robot arm that moves sample tubes back and forth from the tube racks to a weigh station, a filter station, a vortexer station and a homogenization vessel. There are 3-place and 4-place balances to determine sample weight and gravimetrically determine solvent volumes for dilution purposes. At the back of the instrument is a bank of syringes for sample and solvent delivery. An inline filter assembly is located between the homogenizer and the 4-place balance. At the end of the sequence of components is an Easyfill collection module that

keeps the finished sample in a sealed LC vial until needed. All operations are controlled through a PC that also generates a spreadsheet during every run that tracks all pertinent weights, volumes and speeds as well as any errors that may have occurred in the course of the run.

The lengthy description of the automated procedure that follows compared to the brief manual procedure description already discussed can be misleading. This does not equate to more work for the analyst when using the automated workstation. The following procedures need only be entered into methods on the TPWII once using a windows based, menu driven program and then recalled when needed. The analyst only loads the workstation with samples, solvents, sample tubes, LC vials and filters; the workstation will handle everything else.

2.2.1. Sample preparation

The sample preparation for the composite assay and the degradation products samples proceeds as follows. Depending on tablet strength, the required number of tablets are transferred into separate sample tubes in the input rack. An empty tube is placed in the first position, at every 20th position and at the end of the set of samples to be processed. The output racks are filled with the same number of sample tubes as are in the input rack. The automated method uses Millipore Automation Certified™, 0.45 μm nylon membrane filters. Solvents should be prepared and allowed to equilibrate to the temperature of the room where the instrument is located before use to avoid density changes that could cause errors in the gravimetric dispensing.

In following the method program to prepare a sample, the TPWII performs a variety of steps:

In Step 1, the TPWII dispenses dilution solvent into the homogenizer vessel. Solution dispensing is performed gravimetrically based upon previously entered solution densities.

In Step 2, the tablets in the sample tube are transferred into the homogenization vessel by the robot arm and a tipper assembly. After first transferring the sample tube to the 4-place balance to record sample weight, the robot arm places the sample tube in the tipper assembly. The tipper assembly then rotates over the mouth of the homogenizer vessel and shakes in order to release any tablets that might be trapped in the sample tube. At this point the tablets will all be in the homogenizer vessel.

In Step 3 and step 4 of the method, the homogenizer vessel is raised up so that the homogenizer probe enters the solution in the vessel. The homogenizer is then activated, with the probe rotating at 10 000 revolutions per minute for 25 s. This homogenization pulse is repeated six times for each step.

In Step 5, the solution is allowed to soak/settle for 30 s to allow excipient particulates to settle.

In Step 6, the robot arm first places a new filter in the filter holder. Pre-wetting with homogenate then occurs to properly flush and condition the lines leading to the tube from the output rack that the robot arm has concurrently placed in the 4-place balance where the actual sample aliquot of filtered homogenate will be routed. A 5.0 ml sample aliquot of homogenate is then aspirated from the vessel and passed through the filter to remove particulates. The filtrate is dispensed into the tube from the output rack, and the tube is weighed to confirm that at least 90% of the requested volume of filtrate has been collected.

In Step 7, a 1:2 ratiometric dilution is performed. A ratiometric dilution means that an appropriate volume (in this case approximately 5 ml, automatically adjusted depending on the volume of filtrate collected) of dilution solvent is added directly to the filtrate in the output tube to achieve the desired final dilution ratio. This gives a final volume of approximately 10.0 ml.

In Step 8, the output tube is placed in the vortexer station by the robot arm, where it is agitated for 25 s after which it is replaced in the output rack.

In Step 9, 3.5 ml of the assay sample is sent to the Easyfill rack and collected in a sample vial immediately after the path to the easyfill has been conditioned with 4.0 ml of the same sample solution. The material present in the vial at this point is the sample to be used for HPLC analysis.

In Step 10, the TPWII performs clean-up routines to prepare for the next sample. The homogenizer vessel is evacuated. Then it is washed once with 100 ml of dilution solvent. This wash, which is sent to waste, removes excipient particulate material and residual drug remaining in the homogenizer vessel. A second wash is then performed using 500 ml of water from a 20 l reservoir. This water wash is then recycled back to the 20 l wash reservoir. A final wash is then performed using 100 ml of dilution solvent again. The filter transfer path is also washed, using dilution solvent.

The content uniformity sample preparation basically duplicates the preceding steps with the exception of using only one tablet per sample preparation for a total of ten preparations, with solvent volumes adjusted accordingly. The rinse volumes using the dilution solvent are the same as in the assay method, however, only 150 ml is needed for the water rinse since the vessel never contains more than 75 ml of solution during a content uniformity sample preparation. The individual weights for each of the ten samples appear on the spreadsheet generated during the run, having been recorded automatically on the 4-place balance by the TPWII.

2.2.2. Standard preparation

The standard preparation using the automated workstation is very similar to the sample preparation just described, with a few variations. To start, approximately 46 mg of reference standard is accurately weighed directly into the homogenization vessel and placed on the 3-place balance of the TPWII. This is done because the drug substance will not pour cleanly from a sample tube, which would result in inaccuracies in the final solution concentration. During the course of the run an empty sample tube will be tipped over the vessel (even though the drug is already there) because limitations in the controller software make this step a required part of any TPWII method. The soak/settle step is longer, 5 min compared to 30 s, than in the sample preparation to allow the larger amount of drug substance to completely dissolve. Also, a 1:4 dilution of the filtrate with dilution solvent is performed in the standard preparation to achieve the proper final concentration.

The 1% degradation products standard is prepared with a 1:100 manual dilution of the automated assay standard in dilution solvent.

2.2.3. Sample analysis

All Roxifiban samples, whether prepared manually or on an automated workstation, are analyzed as follows. Composite assay and content uniformity samples utilize isocratic reversed-phase LC with UV detection, whereas the degradation samples utilize gradient reversed-phase LC with UV detection.

2.2.4. Validation experiments

The following experiments were performed to demonstrate that the robotic sample preparation method was suitable for its intended purpose [3].

Spiked placebo samples were prepared and analyzed at the following strengths: 80% of 0.5 mg (4.0 mg of Roxifiban total), 100% of 1.0 mg (10 mg of Roxifiban total) and 120% of 2.0 mg (24 mg of Roxifiban total) tablets. These experiments illustrate whether quantitative recovery is possible from the mixture of drug substance and excipients in placebo tablets over a range that exceeds the expected range of sample strengths. Due to the low strengths of the tablets involved it was necessary to introduce the individual spikes as a pipetted aliquot from a prepared bulk solution of Roxifiban in dilution solvent; it would be impossible to weigh Roxifiban powder accurately enough in the small quantities required for the lower concentration spiking experiments. To assure the viability of this approach as opposed to individually weighing each spike in solid drug substance form, three spikes were prepared as individually weighed drug substance samples for comparison. This was done at the highest concentration (120% of 2.0 mg) where there would be an acceptable degree of accuracy in the sample weighing.

A cross-validation study was performed in order to determine whether the automated method produces results which are equivalent to those produced using the validated manual method. Triplicate preparations were made of tablets from four different batches of Roxifiban drug product

at four strengths, 0.5, 1.0, 1.5 and 2.0 mg, using the automated method. The composite assay results obtained using these preparations were compared to the same lots of tablets assayed using the manual method. The data were compared using a Westlake statistical analysis [4,5].

A cross-validation study was also performed to compare impurity determinations by the manual and automated methods. Using the same automated and manual preparations described in the preceding paragraph, four impurities (XV450, SJ459, RRT 1.74 and RRT 2.53) were quantified for the purpose of this validation and their amounts compared.

A final cross-validation study was conducted to compare content uniformity and weight variation measurements performed manually versus the automated workstation. Ten single tablet preparations were made on the TPWII using both 1.0 and 1.5 mg tablets. These results were then compared to data generated using the manual method.

Since the TPWII repeatedly uses the same homogenizer vessel and transfer lines, the effectiveness of the wash procedure was validated by determining carryover. This was done by measuring the amount of Roxifiban in blank samples run after the preparation of a series of 2.0 mg tablets, the highest dosage strength. The sequence run was blank, 20 samples, blank. The amount of Roxifiban in each blank was assayed to determine how much carryover was occurring.

3. Results and discussion

3.1. Method development

In order to create a workable automated procedure, several issues needed to be resolved during method development. The first was to find proper homogenization and dilution volumes that would completely solubilize the active drug while also generating a minimum amount of solvent waste. There also needed to be a sufficient volume of sample solution available after the dilution to properly condition the flow lines and Easyfill loop and supply the LC vial. To best meet these requirements, the initial homogenization step was made with half the solvent volume used in the manual method, and then a subsequent dilution of the filtrate was made utilizing the ratiometric dilution function on the TPWII. This sequence allowed for a minimum use of solvent, full recovery of the drug and ample volume ($\approx$ 10 ml) after the dilution for line washing and sample collection.

The second issue involved finding the proper pre-wet volume to condition the loop between the homogenizer and the output tube to achieve quantitative recoveries. The pre-wet volume is simply the slug of homogenate that is sent through the system tubing to flush and condition the filter and flow path to the output tube. Low recoveries were found to occur if there was inadequate rinsing of this path. As a result of some affinity Roxifiban had for the tubing or the filter, an initial pre-wet volume of 5 ml was necessary to prepare the lines for complete transfer of the filtrate sample. This is considerably more than the 1.5 ml pre-wet volume that is the default in this instrument.

The last concern to be dealt with was finding a compromise in the vessel clean-up step that gave minimum carryover of the drug and maximum efficiency in solvent use. The final sequence consisted of an initial rinse with 100 ml of sample solvent, the minimum volume allowed by the instrument, to remove the majority of the excipient particulates and residual drug. This was followed by a 500 ml rinse with water, the maximum volume allowed by the instrument, to thoroughly clean the whole of the vessel and the homogenizer probe. The large volume of this second wash ensures that the entire height of the homogenizer vessel and probe are adequately cleaned; recycling this large volume wash significantly reduces the amount of waste solvent generated while still allowing adequate washing to achieve acceptably low carryover. A final 100 ml rinse with sample solvent was made to condition the vessel and transfer lines. The solvent rinses were sent to waste, but the water rinse was recycled into the 20 l wash reservoir with no effect on carryover after extended sample preparations. This rinsing regime reduced by 300 ml per sample the amount of mixed aqueous/organic solvent waste produced.

Table 1
Results of accuracy study for roxifiban spiked into placebo

Amount of Roxifiban	Mean	% RSD
4.0 mg[a]	99.9	0.5
10.0 mg[a]	101.0	0.1
24.0 mg[a]	100.3	0.3
24.0 mg[b]	100.0	0.4

[a] Liquid spike
[b] Spike with Roxifiban powder.

Table 2
Cross-validation data for composite assay of 0.5, 1.0, 1.5 and 2.0 mg tablets

	% Label		
	Manual results	Automated results	Difference
0.5 mg tablet			
Mean	100.3	101.1	0.8
RSD	0.9	1.1	
1.0 mg tablet			
Mean	101.0	100.9	0.1
RSD	0.6	0.9	
1.5 mg tablet			
Mean	99.1	99.6	0.5
RSD	0.9	0.3	
2.0 mg tablet			
Mean	99.9	99.3	0.6
RSD	0.4	0.2	

3.2. Validation

3.2.1. Accuracy

The results of the spiking experiments (Table 1) indicate that quantitative recovery of Roxifiban is possible over a range of strengths exceeding that normally found in Roxifiban tablets. The individual values determined vary from 99.4 to 101.1% of the expected value. Precision is also adequate, with an RSD of 0.5% or less. The method precision was not significantly different at the lower and upper limit of the intended range as determined by a two-tailed F-test ($F = 1$; $F_{critical} = 39$, $P = 0.05$). It can be seen that the liquid and powder spike at 24 mg give essentially identical results, giving confidence in the lower level liquid spikes.

3.2.2. Composite assay: comparison of manual and automated methods

Data comparing the automated method with the manual method for assay are presented in Table 2.

The manual and automated results for composite assay of Roxifiban tablets at 0.5, 1.0, 1.5 and 2.0 mg strengths were compared using a Westlake statistical analysis. A 95% Westlake interval was calculated to compare the manual and automated methods, combining data from all four strengths. The calculated Westlake interval of 0.7% indicates excellent agreement between these sets of data.

3.2.3. Impurity determination: comparison of manual and automated methods

Figs. 2 and 3 show chromatograms of impurities in Roxifiban tablets prepared with the automated and manual methods. The similarities between them are clear. Cross-validation for impurities is detailed in Table 3. It can be seen from the results in this table that the levels determined for the impurities XV459, SJ459, RRT 1.74 and RRT 2.53 are very similar using either the manual or automated methods.

3.2.4. Content uniformity and weight variation: comparison of manual and automated methods

Data comparing the automated method with the manual method for content uniformity and weight variation are presented in Table 4. The results of this study show good agreement between the recovery and weight data for the automated and manual methods. When weight data is collected, it is preferable to use the small sample tube rack so the more accurate 4-place balance can be used. Also, since the large sample tube rack forces the use of the 3-place balance located under the homogenization vessel, there is the possibility of residual solvent on the probe falling into the vessel. Even though this extra material is an insignificant change to the

actual dilution volume, it can adversely effect the sample weight determination.

3.2.5. Carryover study

The initial blank, which showed no sign of the drug, and the final blank were collected at the Easyfill and analyzed for Roxifiban. The results of the carryover study showed a minimal 0.07% carryover after 20 composite sample preparations of the highest strength tablets.

4. Conclusion

In the same way that the use of autosamplers for LC has increased efficiency in sample analysis, automated workstations have the potential to make an equal contribution in the area of sample preparation. As shown in the case of Roxifiban tablets, an automated sample preparation method can prove to be a suitable alternative to a manual method while providing additional benefits in the

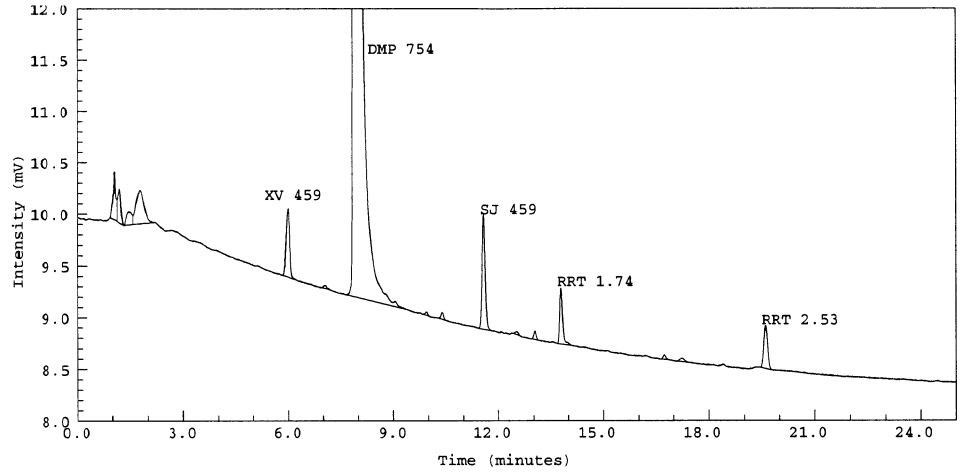

Fig. 2. Chromatogram showing the related products analysis of Roxifiban (DMP 754) prepared using the TPWII.

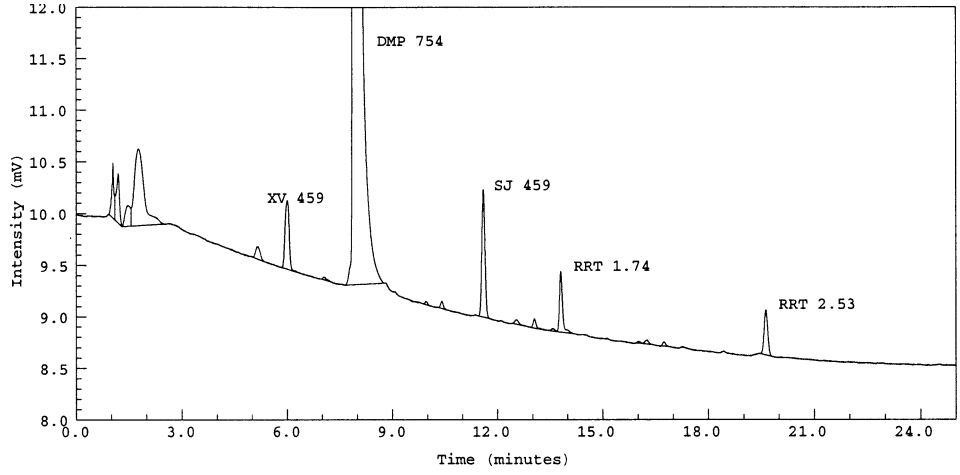

Fig. 3. Chromatogram showing the related products analysis of Roxifiban (DMP 754) prepared using the manual method.

Table 3
Comparison of determinations of the XV459, SJ459, RRT 1.74 and RRT 2.53 impurities by automated and manual methods

Mean determined impurity	0.5 mg tablet (%)	1.0 mg tablet (%)	1.5 mg tablet (%)	2.0 mg tablet (%)
XV459: automated method	0.15	0.22	0.19	0.21
XV459: manual method	0.14	0.23	0.17	0.20
SJ459: automated method	0.30	0.33	0.29	0.29
SJ459: manual method	0.29	0.32	0.28	0.28
RRT 1.74: automated method	0.13	0.13	0.13	0.13
RRT 1.74: manual method	0.14	0.14	0.13	0.13
RRT 2.53: automated method	0.13	0.13	0.13	0.13
RRT 2.53: manual method	0.14	0.13	0.12	0.12

Table 4
Comparison of content uniformity and weight variation by automated and manual methods

	1.0 mg tablet (% label)	Weight (mg)	1.5 mg tablet (% label)	Weight (mg)
Automated method				
Mean	100.5	123.0	98.3	183.0
High value	105.7		101.9	
Low value	97.4		95.3	
RSD	2.2	0.6	1.9	0.5
Manual method				
Mean	99.2	123.0	99.5	184.0
High value	102.3		101.6	
Low value	96.2		96.8	
RSD	2.1	0.7	1.6	0.3

process. While not completely problem free, transfer of a manual method to an automated system does become quicker and less problematic as familiarity with the instrument and its limitations increases. The validation requirements involved relative to the automated method tend to be less extensive than the previous work necessary to validate the original manual method.

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