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Transfer from manual to automated sample preparation: a case study

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Abstract

A manual sample preparation for a controlled release capsule formulation has been converted to an automated sample preparation. Each step of the manual sample preparation was evaluated as to its feasibility for automation in terms of precision, carryover, filter selection and other critical issues. Although most steps of the manual method were easily translated to the automated procedure, certain 'simple' details such as filter selection, sample storage, and the conversion from volumetric to gravimetric measurements needed closer investigation © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

In the last decade, laboratory robots have been increasingly used in the analysis of pharmaceutical dosage forms. One advantage is the significant reduction in labor-intensive but routine activities in an analytical laboratory. Because of the potential cost savings, many pharmaceutical companies are following the trend toward automation [1]. The major applications in the pharmaceutical field are dissolution testing [2–4] and content analyses [5,6] which include content uniformity and composite assays. For a compound that has been approved and commercialized, the most common routine but labor-intensive analyses are the content uniformity and in-process blend uniformity tests. In contrast, if a compound is in Phase III clinical study, the most labor-intensive analysis is the composite assay for stability evaluation.

To increase sample throughput and improve analytical precision in assay determinations, many laboratories are switching from manual sample preparation to automated sample preparation using laboratory robots. Automation increases precision by improving the mechanical consistency of all steps in the sample preparation process. It also permits unattended operation, thereby freeing laboratory personnel to design and perform other experiments that increase overall productivity. Although robots can be used for performing assays

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in the early development of drugs, the greater resource savings lie in routine stability assays during Phase III clinical studies or after drug approval. The initial investment of money and time for automated methods are small when compared to the savings from increased reproducibility, higher throughput and independent operation. An additional advantage is that automated sample preparation minimizes exposure of laboratory personnel to biologically active compounds, especially those that are highly potent.

The work described herein was focused on an analytical method for a pharmaceutical product in Phase III clinical studies for which data were being generated to support a new drug application (NDA). The compound is a phenothiazine derivative with pK_a of 3.4 and 7.8. The compound is very slightly soluble in water but somewhat more soluble in methanol (0.4 mg/ml).

The manual method required tedious and time-consuming laboratory operations. The goal was to convert an existing and validated manual sample preparation for the HPLC assay to an automated procedure using a Zymark TPW II® (Tablet Processing Workstation). As is common in many laboratories, no thought had been given to conversion to an automated method when the manual method was originally developed.

This case study is focused on the operations involved in converting a validated manual sample preparation method to an automated method. The results are presented with regard to precision, carry-over, filter selection and other critical problem areas.

2. Experimental

2.1. Tablet Processing Workstation (TPW II®)

The automated sample preparation was performed on a Tablet Processing Work Station (TPW II®, Zymark, Hopkinton, MA). The TPW II® incorporates a four-place analytical balance, a three-place top loading balance, a homogenizer, a membrane filtration unit, and an EasyFill® module. This module allows samples from the test tubes in the TPW II® to be transferred into capped vials for off-line sample analysis. The EasyFill® module is sold as optional equipment for the TPW II®. All operations were controlled by a personal computer.

All sample preparation steps, including weighing, extraction, transfer, filtration, and cleaning of the vessel, were performed by the instrument. The work station program recorded all sample information, including weights and transferred volumes, in the form of a Microsoft Excel® spreadsheet.

2.2. Manual sample preparation

Twenty-five capsules were opened and the contained pellets were combined. The empty capsule shells were discarded. The combined pellets were weighed and the average fill weight in mg/capsule was determined (total pellet weight/number of capsules). The pellets were ground to a fine powder using either a mortar and pestle or a Spex 8000 Mixer/Mill (Spex Industries, Edison, NJ), for approximately 2 min. An accurately weighed aliquot of powder equivalent to 60 mg of active drug substance was then transferred to a 250-ml volumetric flask. This step was performed in triplicate for each sample.

Approximately 150 ml of methanol was added to the 250-ml volumetric flask. The mixture was sonicated for 15 min and then placed on a platform shaker for 15 min. The shaker was set to approximately 200 cycles per min. The shaken mixture was diluted to volume with methanol and mixed well. A portion of the solution was filtered through a 0.45-μm Gelman Acrodisc® PTFE syringe filter, discarding the first few milliliters. An aliquot of this filtrate was then transferred to a suitable HPLC vial. Total sample preparation time was 75 min.

2.3. Automated sample preparation using TPW II®

Four 15-mg strength or six 10-mg strength capsules were opened and their contents were transferred to a test tube. The capsule shells were discarded. Methanol was added to the vessel followed by automated pellet transfer from the test

tube to the vessel using the tipping arm set at 'speed 1'. After the transfer was complete, the test tube was shaken at 'speed 3' for 2 s to ensure all pellets were added. The pellets were homogenized using six 20-s pulses at 10 000 rpm followed by two 20-s pulses at 12 000 rpm. The solution was allowed settle for 15 s. The homogenizer and the attached transfer line were raised such that the opening of the line was at 70% of the liquid level. This served to minimize plugging of the filter and the transfer line by suspended solids. The filter was prewetted with 3.0 ml of the solution and 10.0 ml of homogenate was filtered at 0.10 ml/s. The Easyfill transfer line was conditioned twice with 2 ml of filtrate and a total of 1.5 ml of sample was transferred to an 11-mm amber HPLC vial. The method ended and the clean-up routine began. The homogenization vessel was washed once with 100 ml of methanol and the filter transfer path was washed twice with 3 ml of methanol. The Easyfill transfer path was washed twice with 4 ml of methanol and the needle was washed with methanol. Total sample preparation time was 18 min.

2.4. High performance liquid chromatography (HPLC)

All HPLC experiments were carried out with an HP 1050® high performance liquid chromatography system (Hewlett-Packard, Palo Alto, CA) with a UV detector at 254 nm. The data acquisition system was a Nelson System 6000® with Nelson Access Chrom (Version 1.8). A Waters MicroBondapak® (Milford, MA) C-18 column (3.9 mm × 300 mm, 10 μm particle size) was used. The mobile phase composition was sodium octanesulfonate (0.02 M)/acetonitrile/methanol (47:48:5). The flow rate was 1.5 ml/min at ambient temperature and the injection volume was 15 μl. Sodium octanesulfonate was obtained from Sigma (St Louis, MO), and used without purification. Acetonitrile and methanol were HPLC grade. Deionized water was filtered through a Nanopure water purification system from Barnstead/Thermolyne (Dubuque, IA). The standard solutions used in both the manual and the automated sample assays were prepared by the manual procedure.

3. Results and discussion

The pharmaceutical capsules used in this study contained either 10 or 15 mg of active component. These capsules were composed of a mixture of immediate release pellets, sustained release pellets and colored sugar spheres packed in a hard gelatin capsule. The immediate release and sustained release pellet fill weights were proportional for the 10- and 15-mg capsules. The sustained release pellets were coated with non-disintegrating polymers. Therefore, it was difficult to extract the active ingredient without grinding or breaking the coating prior to the analysis. As will be discussed later, the excipients also made sample filtration very difficult.

In the manual method, the capsule shells were opened by the analyst and the shells discarded. In the automated method, the capsule shells could simply have been crushed into tiny pieces during the homogenization step. However, in this case, several months of stability analyses had previously been performed using the manual method in which the capsules shells were discarded. There was the remote possibility of an interaction between the shells and the drug substance in methanol, which might compromise comparisons between data obtained by the manual and automated methods. It was decided to continue discarding the shells in the automated method.

As discussed in Section 2, the manual sample preparation required at least 75 min for complete extraction from the pellets, as compared to 18 min for the automated procedure. Since a previously validated manual sample preparation was being converted to an automated procedure, only the following steps required validation: (1) extraction, (2) filtration, and (3) carry over. Although it was not necessary for this method, validation of any dilution steps may also be needed in other applications.

While virtually any amount of extraction solvent can be used in a manual method, there are limitations in the extraction volumes that can be used in the TPW II®. For example, if the compound has a limited solubility, a large volume of extraction solvent could be used in a manual method. However, because of instrument design,

the extraction solvent is limited to 500 ml in the TPW II® method. Thus, for poorly soluble compounds, it may be necessary to identify a better extraction solvent or to reduce sample size and accept the inherently higher relative error. Because of these considerations, it may be easier to develop an automated method using the TPW II® from the beginning than to convert a previously developed manual method.

It should be noted that, in our case, the manual method was based on volumetric measurements while the automated method was based on gravimetric measurements. When the manual method was originally validated, it was not expected to be transferred to an automated method. When an automated method is developed from the beginning, gravimetric measurements are just as simple as volumetric measurements and are actually preferable. However, converting a volumetric method to a gravimetric method can be problematic. For example, when a manual method calls for 250 ml of a solution prepared in a volumetric flask, the procedure is to first add tablets/capsules into the flask and then add the extraction solvent until the target volume is reached. In the automated method, the procedure would be to add 250 ml of extraction solvent into the homogenizer vessel and then add the tablets/capsules or vice versa. These two processes deliver different amounts of the extraction solvent due to the displacement volume of the sample, resulting in a slight error in some cases. To minimize this problem, the volume displaced by the sample was measured and subtracted from the extraction solvent volume. In this way, the automated method and the manual method added essentially the same amount of extraction solvent. An alternative approach would be to gravimetically determine the amount of solvent transferred in the manual method and use this exact amount in the automated procedure.

A homogenization study was performed to determine the efficiency of the extraction under particular experimental conditions. If the pellets broke into fine particles, there were no extraction problems. In contrast, if the pellets fractured into larger pieces, the extraction was less complete. Critical parameters such as homogenization time and speed were established by visual inspection of the homogenate, then optimized based on HPLC results. Three different homogenization speeds were evaluated. In all cases, six 20-s pulses at the first speed followed by two 20-s pulses at the second speed were satisfactory. The middle case (10 000/12 000 rpm) was selected for the automated method. No significant differences in assay were observed between the manual method ($n = 3$, 81.6 mg/g, RSD = 1.8%) and the automated method ($n = 3$, 81.8 mg/g, RSD = 1.6%); nor were there any differences in degradation profiles.

One concern with this particular instrument was that the homogenization efficiency, i.e. homogenizer speed, may change over time due to mechanical wear or may vary from instrument to instrument. This problem could be overcome by using an independent speed check during routine maintenance.

A recovery experiment was performed at approximately 75, 100, and 125% of the assay concentration. The drug substance was spiked in the presence of the excipients. The average recovery values were 99.6% ($n = 3$, RSD = 0.2%) at 75%, 99.6% ($n = 3$, RSD = 0.1%) at 100%, and 99.5% ($n = 3$, RSD = 0.5%) at 125% of the assay concentrations.

Optimization of the filtration step was then performed. When beginning the work on the automated method, it was mistakenly thought that filtration would not be a significant issue. However, this proved to be one of the most problematic steps in the transfer from the manual to the automated method. In the filtering step, three factors are of concern: (1) Particulate material should not pass through the filter, (2) the active substance should not be retained on the filter membrane, and (3) there should be adequate flow through the filter. In the manual method, a Gelman 0.45-μm Acrodisc CR® PTFE syringe filter was used. Although the final sample concentration was identical for both manual and automated methods, the same filter could not be used in the automated method. While it was somewhat difficult to manually force the filtrate through the filter, this was not a problem in the manual method since only 4.5 ml of filtrate was needed (discard first 3 ml, and collect 1.5 ml sample). In

contrast, it proved impossible to obtain the minimum 9 ml of filtrate (discard first 3 ml, 2×2 ml rinses of the Easyfill® tube, and collect 1.5 ml) required by the automated method through these PTFE filters.

When a filter with large pore size (Millipore 1.0-μm glass fiber filter) was examined, some of the particulates passed through the filter membrane. A two-stage Millipore filter (1.0-μm glass filter and 0.5-μm PTFE filter) was also examined. Here, small amounts of unfiltered solids were observed in the filtrate along with high back pressure. A Gelman 0.45-μm GHP Acrodisc® syringe filter seemed to perform well until approximately 10 ml passed through the filter, but there was substantial back pressure after 10 ml. A Gelman 0.45-μm GHP Acrodisc® GF filter was found to remove all solids from the filtrate and offered no back-pressure problems and was selected for this application. Although the filter was not designed specifically for the TPW II®, no problems with leaking or crushing of the filter were observed in our application. This better performing filter was subsequently incorporated into the manual as well as the automated methods.

The test tubes normally used with the TPW II® allowed evaporation of solvents because of the loose fit between the cap and the top of the test tube. This could have introduced error when using volatile solvents such as methanol. Thus, the Easyfill® transfer system was necessary for this automated method. By using the Easyfill®, samples were directly collected in sealed amber HPLC vials.

In order to determine the level of cross-contamination between samples, a carry-over study was performed wherein each sample preparation was followed by a blank solution preparation. Six samples and six blanks were tested. The average carry-over was found to be 0.2%, even with only one 100-ml wash of methanol. However, in other applications where the solubilities of the analytes are lower, a larger wash volume may be needed. If there is a concern about disposal of waste organic solvents, the amount or numbers of washes can be reduced, but at the cost of higher carry-over levels.

In our study, we adjusted the number of capsules of different strengths such that the nominal concentrations of active substance were the same in all runs. This minimized errors arising from carry-over between runs. Carry-over can be

Table 1
Assay results for the automated and manual methods

		Manual method (% label claim)	Automated method (% label claim)
Lot #96006		97.1	97.7
		96.9	96.9
		96.9	97.6
		98.9	99.5
		96.8	99.2
		100.0	97.4
	Average	97.8	98.1
	RSD (%)	1.4	1.1
Lot #96008		100.3	100.0
		99.6	99.4
		99.4	100.3
		100.4	100.6
		100.0	101.2
		100.0	99.7
	Average	100.0	100.2
	RSD (%)	0.4	0.6

more problematic when substantially different concentrations of active substance are examined in the same automated sequence of analyses. In this latter case, the analyst should run a blank (cleanout) sample between samples with different analyte concentrations.

Table 1 shows the comparison of assay results using the manual and automated methods. As can be seen, there is no significant difference in the results between the methods.

4. Conclusion

The automated sample preparation method was shown to be equivalent to the manual sample preparation method. The only labor-intensive work for the analyst is to add samples into the sample tubes, fill the solvent reservoirs, and discard the sample tubes. Thus, compared to traditional manual sample preparation, automated sample preparation offers significant savings in manpower.

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