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# Enhanced robotic dissolution system with concurrent off-line analysis

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**Abstract**: A Zymate™ II robotic dissolution system was modified by addition of a liquid chromatography (LC) system equipped with an Isco ISIS autosampler. Using the Concurrent EasyLab™ programming language, operation of this LC system was integrated into the main robotic dissolution procedure. This enabled simultaneous dissolution sampling and LC analysis of standard and pre-pulled sample solutions. The resulting dual-tasking system effects considerable efficiency of operation and results in significant time savings. Comparison of the robotic and non-robotic data indicate good correlation and statistically insignificant differences at the 95% confidence level. Validation studies confirm linearity and precision of the quantitative LC method, accuracy and precision of volume and temperature measurements and negligible vessel-to-vessel carryover.

**Keywords**: *Dissolution; robotic dissolution; automated dissolution; off-line analysis; concurrent processing.*

## Introduction

Dissolution studies are among the most widely performed tests for solid oral pharmaceuticals and are thus prime candidates for automation. Over the past few years, various approaches have been taken to achieve this automation, as recently reviewed by De Castro and Valcarcel [1]. In general, non-robotic automated dissolution systems achieve only partial automation, such as automation of sample collection and/or analysis [2]. Robotic systems, such as the Zymate™ II Dissolution Robot [3], are capable of automating transfer of the dosage form to the dissolution vessel as well as cleaning of the vessel between dissolution runs. The latter features enable the system to carry out multiple runs.

The Zymate system typically comes equipped with a UV spectrophotometer for on-line sample absorbance measurement, although it can be custom-configured for on-line HPLC or flow injection analysis (FIA) or with racks for sample storage and off-line analysis [4–6]. Systems configured for on-line LC analysis require chromatographic run times that are less than the time interval between sampling from consecutive dissolution vessels [4, 5]. This requirement often precludes use of dissolution robots when rapid profiling of the dissolution curve, such as sampling every 10 min, is required. A recent non-robotic system described by Mathieu *et al.* [7] is equipped with an automatic tablet dispenser to enable multiple runs. However, since the system employs only high speed LC (run times ≤2 min) for on-line sample analysis, adapting an existing procedure to the system requires a new LC method. In another approach described by Kostek *et al.* [8], two full-fledged robots are employed, one for dissolution sampling and the other for sample analysis. The system, dubbed 'the complete dissolution robot', requires significant initial capital outlay as well as a high level of programming expertise to manage the schedules between the two robots. The system described in this report takes advantage of the Zymate II software to create a flexible, fully integrated robotic system with off-line HPLC–FIA analysis. It is capable of directly adapting existing non-robotic dissolution procedures.

## Experimental

### *System design — hardware*

The layout of the robotic system is shown in Fig. 1. The robotic dissolution system consists

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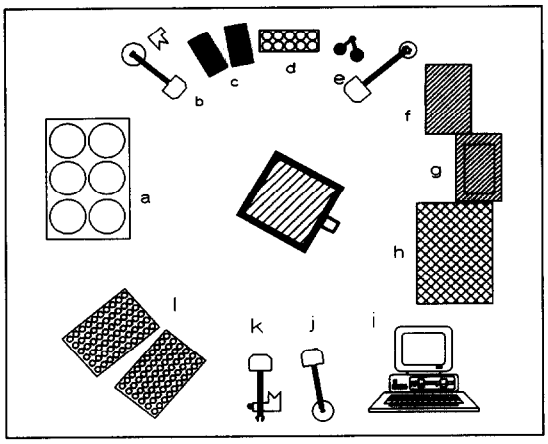


**Figure 1**
Schematic diagram of robotic dissolution system: (a) dissolution bath; (b) sipper fill hand; (c) filter racks; (d) standard rack; (e) general purpose hand II; (f) master laboratory station; (g) ISIS autosampler/injector; (h) HPLC system; (i) system V controller; (j) wash hand; (k) general purpose hand; (l) sample racks.

of a modified Zymate II Dissolution Robot (Zymark Corporation, Hopkington, MA) equipped with a System V controller running version 1.51 of the Zymark Robotic Operating System. The dissolution bath, a Vanderkamp 600 six-unit dissolution tester (Van Kel Industries, Edison, NJ) was fitted with either extended-length rotating paddles (Van Kel Industries) or telescoping basket shafts (Zymark). Sample analysis was done on an HPLC system featuring a modified ISIS autosampler (Isco, Lincoln, NE), Zymark Z310 HPLC Injector Station, Hitachi T6300 Thermostated Column Oven (Hitachi Instruments, Danbury, CT) and ABI models 783 UV Detector and 400 Solvent Pump (Applied Biosystems, Ramsey, NJ). The ISIS autosampler operation is managed by the System V controller through contact closure commands on a Power and Event Controller (PEC) (Zymark). These commands determine the height and lateral position of the sample probe as well as the position of the sample tubes. Delivery of samples to the ISIS tubes and injection into the HPLC is through a two-syringe Master Laboratory Station (MLS) (Zymark) connected to the ISIS sample probe. The HPLC system is interfaced through a Beckman MK5 digimetry unit (Beckman Instruments, Allendale, NJ) to a Beckman Computer Automated Laboratory System (CALS$^{TM}$) running PeakPro$^{TM}$ software.

*Software system design*

The system software features simultaneous dissolution and off-line sample analysis, using Zymark's Concurrent EasyLab$^{TM}$ software. A flow diagram of the operation is shown in Fig. 2. The program flow consists of two simultaneous, independent operations, i.e. sample dissolution and sample analysis. Branching of the main program takes place upon execution of a 'Meanwhile' command which switches the system into dual operation or dual processor mode, i.e. the system controller's microprocessor divides its time between both operations and thus functions as two processors. The work space and requisite hardware for both operations are independent and inter-

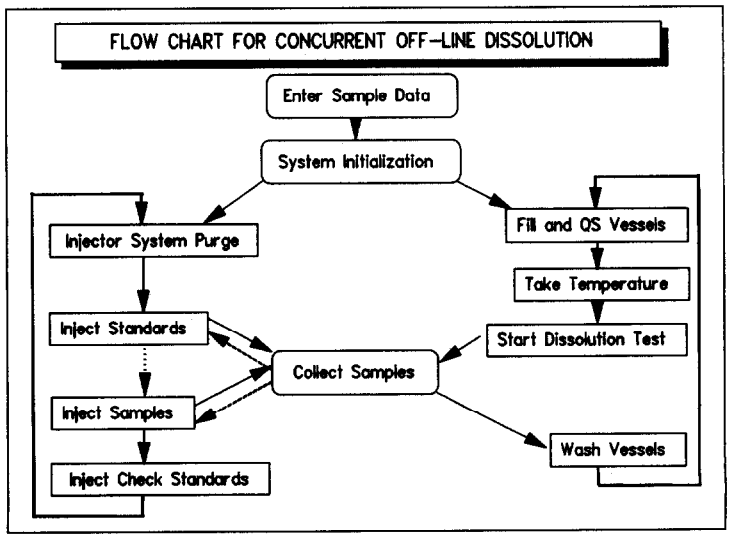


**Figure 2**
Flowchart of dissolution robot operation.

active overlap occurs only when a sample is pulled from the dissolution vessels. The interaction is necessary because the autosampler is common to both operations during dissolution sampling. Timing data for each operation is maintained in independent memory registers in the robot controller. The autosampler timer is used to determine HPLC run time while the dissolution timer is used to set sampling time. Typically, the HPLC run time is shorter than the dissolution run and is thus assigned a lower priority. This means that for each dissolution run, the dissolution system time is continuous while the autosampler time (HPLC run time) is discontinuous. The autosampler is thus programmed to check the value of the dissolution timer prior to each sample injection by executing the following line of program code:

```
IF (SAMPLE.CHECK.TIME -
INJECTOR.TIMER) <
(F * (LC.RUN.TIME+ISIS.CYCLE.TIME+
ISIS.INJECT.TIME)) THEN 210,
```

where SAMPLE.CHECK.TIME = dissolution sampling time, INJECTOR.TIMER = elapsed time since beginning of the dissolution run, F = multiplier to allow for potential delays in the switch from dual processor to single processor mode (values range from 1 to 3), LC.RUN.TIME = run time for each HPLC run, ISIS.CYCLE.TIME = time required for ISIS autoinjector to position next sample tube under sample probe, and ISIS.INJECT.TIME = time for complete sample injection.

Line 210 exits the injector program and resets the robot to single processor mode for dissolution sampling. Upon completion of sampling, the system again checks the timer. If there is adequate time to complete HPLC analysis of the next sample, the concurrent processor mode is resumed at the point of previous suspension. Use of the HPLC run time as a variable enables the system to accept existing HPLC methods without modification.

*System tests and validation*

Three capsule formulations (Types I, II and III) and a tablet formulation were employed to evaluate and validate the system's operation. The test conditions are described in Table 1. The robot switches easily between both methods because it is equipped with two sample racks (a basket rack for capsule dissolution and a plastic cup rack for tablet dissolution) and two general purpose robot hands. Attachment of baskets to the telescoping shafts [5] was automatic, but changeover from basket to paddle shafts was done manually because the system has only one bath. The non-robotic data were generated from dissolution tests performed on the same lots of capsules and tablets using similar dissolution baths. Samples were pulled manually, placed in HPLC vials and analysed. Tests were also carried out to validate critical processes in the robot operation and estimate the efficiency of the system.

## Results and Discussion

*Capsule dissolution*

Comparison of the robotic and non-robotic capsule data (Figs 3–5) indicate good agreement among the three data sets. Repeated measures analysis of variance of the capsule data set (Table 2), using the statistical software 'SOLO' (BMDP, Los Angeles, CA) indicates that the effect of dissolution method (non-robotic or robotic) on per cent drug dissolved is negligible ($F = 0.12$, $P = 0.7278$). The overall mean difference between robotic and non-robotic data was about 1%. This difference is insignificant in view of the intra-method variation observed for each lot of the product (see % RSD data in Table 3).

In terms of sampling position and timing, the accuracy and precision of the robot is superior to the manual method. On this basis, the robotic data would be expected to be more precise, i.e. lower % RSDs for sample replicates. The data in Table 3 indicate that this is generally true, although the difference between the two methods is not significant. This may reflect differences in variance of the two data sets, especially since the number of replicates are different for both methods (non-robotic: $n = 12$; robotic: $n = 6$). In addition, as indicated by Papas *et al.* [3], the variability inherent in the capsule dissolution process may be more significant than that resulting from the dissolution methodology.

*Tablet dissolution*

The non-robotic tablet dissolution data consisted of only averaged 120 min data points, hence comparisons were made only with equivalent single point robotic data (Table 4). Student's *t*-tests on the robotic and non-robotic mean per cent dissolved data at 120 min

**Table 1**
Dissolution and LC analysis parameters for test capsules and tablets

| Parameter | Capsule dissolution | Tablet dissolution |
|---|---|---|
| **Dissolution parameters** | | |
| Apparatus | USP 1 (baskets) | USP 2 (paddles) |
| Rotational speed | 100 rpm | 50 rpm |
| Medium (900 ml at 37°C) | Purified water | 0.05 M Tris(hydroxymethyl)methane (pH 9.0) + 1% sodium lauryl sulphate |
| Sampling times | 30, 60, 120 min | 60, 90, 120 min |
| **LC parameters** | | |
| Column | Hewlett–Packard ODS 100 mm × 2 mm, 5 μm at 40°C | |
| Mobile phase | Methanol–distilled water–glacial acetic acid (40:60:0.5) at 1.0 ml $min^{-1}$ | |
| Detector wavelength | 254 nm | |
| Injection volume | 20 μl | |

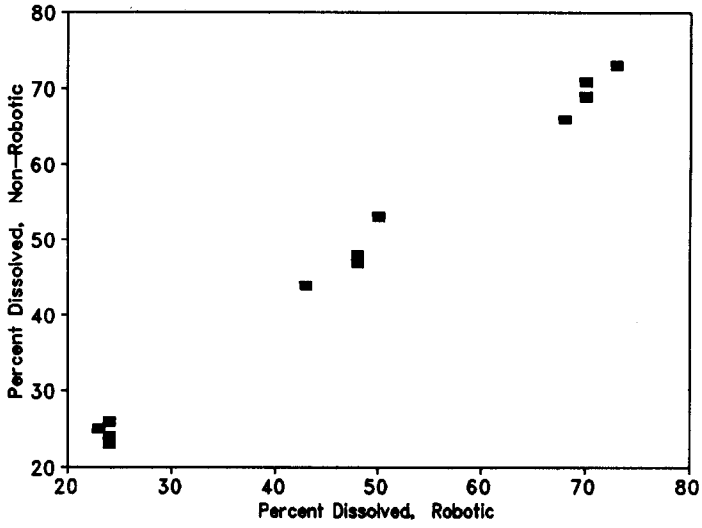


**Figure 3**
Scatter diagram of robotic and non-robotic per cent dissolved data for Type I capsules. Correlation coefficient = 0.9974.

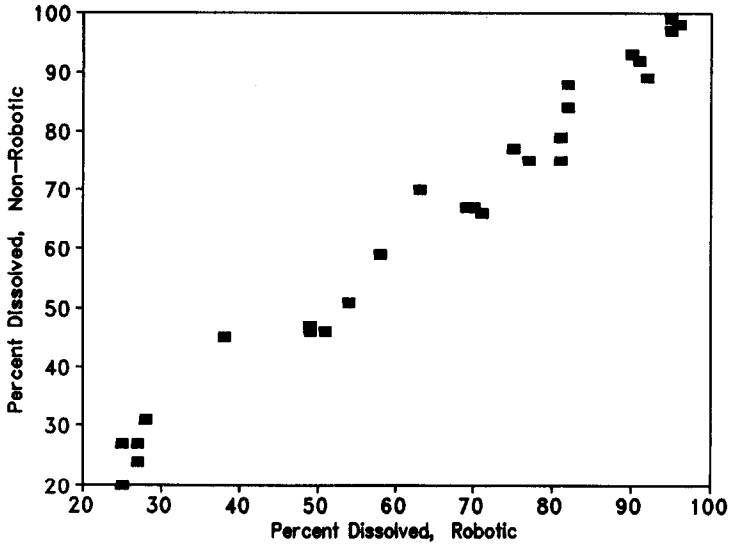


**Figure 4**
Scatter diagram of robotic and non-robotic per cent dissolved data for Type II capsules. Correlation coefficient = 0.9890.

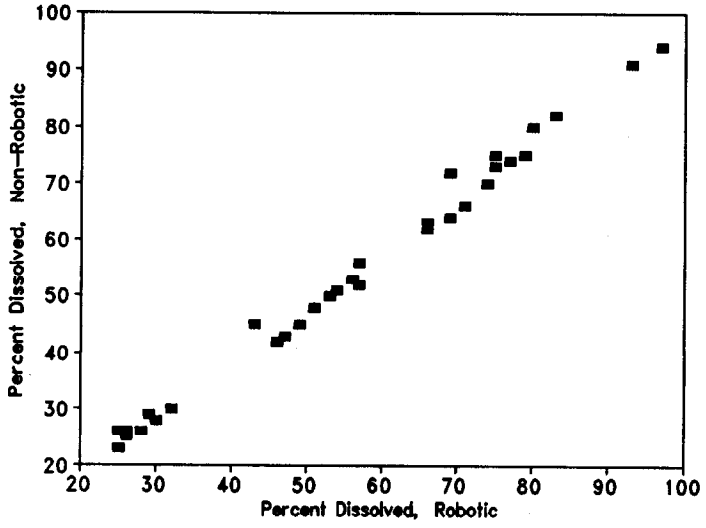


**Figure 5**
Scatter diagram of robotic and non-robotic per cent dissolved data for Type III capsules. Correlation coefficient = 0.9959.

**Table 2**
Repeated measures analysis of variance for capsule dissolution data

| Source | d.f. | Sum of squares | Mean square | *F*-ratio | Prob. > *F** | Error term |
|---|---|---|---|---|---|---|
| Method (*A*) | 1 | 82.42773 | 82.42773 | 0.12 | 0.7278 | S (*A*) |
| S (*A*) | 46 | 30913.57 | 672.0341 | | | none |
| Sample time (*B*) | 2 | 42044.43 | 21022.21 | 800.08 | 0.0000 | error |
| *A* × *T* | 2 | 3.597779 | 1.79889 | 0.07 | 0.9339 | error |
| Error | 92 | 2417.305 | 26.27505 | | | |
| Total (Adj.) | 143 | 75461.33 | | | | |

*At 95% confidence level, a probability value ≤0.05 indicates factor significance.

**Table 3**
Comparative per cent relative standard deviation (% RSDs) for robotic and non-robotic dissoution data

| | | Sampling time | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 30 min | | 60 min | | 120 min | | |
| Capsule type | Dissolution method | Mean | Range | Mean | Range | Mean | Range | Overall mean % RSD |
| Type I | Robotic | 7.8 | 5.9–10.7 | 5.8 | 5.4–6.4 | 4.6 | 3.8–5.2 | 6.0 |
| | Non-robotic | 12.5 | 10.4–17.2 | 6.4 | 4.8–11.0 | 5.1 | 2.9–9.3 | 8.0 |
| Type II | Robotic | 6.4 | 1.9–19.3 | 4.6 | 1.9–12.1 | 3.7 | 1.9–7.2 | 4.9 |
| | Non-robotic | 11.5 | 5.4–30.6 | 6.3 | 3.5–17.1 | 3.4 | 1.2–6.6 | 7.1 |
| Type III | Robotic | 10.5 | 4.9–24.4 | 5.7 | 1.9–13.2 | 3.9 | 1.9–6.2 | 6.7 |
| | Non-robotic | 11.8 | 4.6–30.6 | 7.6 | 3.2–20.2 | 5.0 | 2.4–14.5 | 8.1 |

**Table 4**
Robotic and non-robotic dissolution of test tablets

| | Robotic | | | | | | Non-robotic | |
|---|---|---|---|---|---|---|---|---|
| | 60 min | | 90 min | | 120 min | | 120 min | |
| Serial lot number | % *D* | % RSD | % *D* | % RSD | % *D* | % RSD | % *D* | % RSD |
| 1 | 52 | 20.9 | 76 | 13.4 | 90 | 6.0 | 93 | 7.1 |
| 2 | 69 | 11.1 | 91 | 3.6 | 98 | 2.8 | 101 | 3.5 |
| 3 | 52 | 21.7 | 79 | 11.3 | 93 | 4.8 | 94 | 9.7 |
| 4 | 74 | 17.0 | 96 | 4.3 | 101 | 2.4 | 99 | 3.9 |
| 5 | 47 | 20.8 | 72 | 11.9 | 84 | 4.8 | 87 | 14.9 |
| 6 | 50 | 24.0 | 78 | 14.0 | 93 | 3.6 | 93 | 10.0 |
| 7 | 72 | 19.9 | 96 | 5.2 | 100 | 3.0 | 97 | 6.0 |
| 8 | 67 | 25.0 | 93 | 7.5 | 99 | 3.0 | 98 | 3.2 |
| 9 | 75 | 17.3 | 98 | 3.5 | 102 | 2.4 | 95 | 9.6 |
| 10 | 53 | 32.6 | 79 | 14.8 | 94 | 8.9 | 86 | 14.4 |

indicates that the intra-lot difference was not significant ($t = 0.87297$; $P = 0.4054$). As was observed with the capsules, the robot data was slightly more precise than the non-robotic data. However, dissolution of the test tablets was found to be sensitive to the location of the tablet in the vessel, the tablets dissolving at different rates depending on how eccentric they were from the bottom centre of the vessel. This sensitivity to hydrodynamics could explain the relatively high % RSDs obtained in the study as well as the insignificant difference between the precision of the robotic and non-robotic data.

*Linearity and precision of the HPLC method*

The HPLC procedure was previously validated for non-robotic use. Hence, only limited validation was carried out on the robot. Linearity and precision of the analytical system was evaluated by replicate injections of a series of standard solutions of the drug in a concentration range of 25% to 122% *D* for capsule and tablet dissolution (*D* is the concentration obtained upon 100% dissolution of the label claim of drug per tablet or capsule). Regression analysis of the mean peak area versus drug concentration data indicate a linear relationship (coefficient of determination, $r^2 = 0.99998$). Precision of the HPLC system operation is demonstrated by the low % RSD for replicate injections (range of % RSDs for six replicate injections of each sample = 0.08–1.23%).

*Sample carry-over*

Carry-over of samples from vessel to vessel could occur with the robot since the system uses the same probe ('sipper.fill.hand') to pull samples from all vessels. Two experiments were carried out to investigate vessel to vessel sample carry-over. Capsules were placed in baskets 1, 3 and 5 to observe any presence of carry-over into vessels 2, 4 and 6, which were without any capsules. The procedure was repeated placing capsules into baskets 2, 4 and 6. Tables 5 and 6 contain summaries of the results, expressed as per cent of the label claim of drug dissolved found in each vessel at each sampling time. The data indicate negligible vessel-to-vessel carry-over.

*Validation of temperature measurements*

The robotic software was written to prevent commencement of dissolution and sample analysis prior to successful measurement of vessel temperatures (37°C ± 0.5°C). To verify accuracy of robotic thermistor measurements, temperature readings of vessel contents were taken using a NIST calibrated thermometer simultaneous with the robotic thermistor measurement. The data obtained (Table 7) show that there is no significant difference between temperatures measured by either method.

**Table 5**
Sample carry-over data for robotic dissolution (% dissolved). Vessels 2, 4 and 6 are without capsules

| | Vessel number | | | | | |
|---|---|---|---|---|---|---|
| Time (min) | 1 | 2 | 3 | 4 | 5 | 6 |
| 30 | 23 | 0 | 23 | 0 | 26 | 0 |
| 60 | 42 | 0 | 42 | 0 | 42 | 0 |
| 120 | 61 | 0 | 60 | 0 | 57 | 0 |

**Table 6**
Sample carry-over data for robotic dissolution (% dissolved). Vessels 1, 3 and 5 are without capsules

| | Vessel number | | | | | |
|---|---|---|---|---|---|---|
| Time (min) | 1 | 2 | 3 | 4 | 5 | 6 |
| 30 | 0 | 23 | <0.2 | 26 | 0 | 27 |
| 60 | 0 | 41 | 0 | 43 | 0 | 42 |
| 120 | <0.2 | 59 | 0 | 60 | 0 | 57 |

**Table 7**
Temperature validation data

| Vessel no. | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Robotic temp. (°C) | 36.7 | 37.1 | 37.2 | 37.3 | 37.4 | 37.4 |
| Manual temp. (°C) | 36.6 | 37.1 | 37.3 | 37.2 | 37.4 | 37.4 |

**Table 8**
Data for validation of volumetric 'QS' procedure

| Vessel no. | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Volume as % of stated volume | 99 | 98 | 103 | 103 | 99 | 102 |

*Validation of volumetric 'QS' procedure*

The robotic system delivers the specified volume (900 ml) of dissolution medium into each vessel by a 'QS.HEIGHT' procedure, involving overfilling the vessels followed by aspiration of the excess medium to pre-taught heights representing the stated media volume. To validate this procedure, programs were executed to simulate media delivery into the vessel. Aliquots of the medium were then removed from each vessel and replaced with an equal volume of a stock standard solution. The solution was stirred, sampled and assayed by HPLC against a manually diluted standard of similar concentration. The concentration obtained was expressed as a per cent of the expected volume of dissolution medium (Table 8). The data indicate accuracy of the volumetric 'QS' procedure. Although not used on this system, a currently available gravimetric vessel fill accessory (Zymark) ensures greater accuracy of the vessel fill procedure.

*System efficiency*

In a recent article on laboratory automation, Isenhour *et al.* [9] indicate that a laboratory robot takes longer to perform a single non-routine task than a skilled technician. Hence, the time advantage of a robotic operation results from completion of several consecutive runs. For this robotic dissolution system, the time required to carry out several steps in the dissolution procedure were determined by setting timers in the robot controller. This was done for both concurrent and non-concurrent operational modes. For comparison, reasonable estimates of the time required by a trained analyst to carry out the same steps were made (Table 9 and Fig. 6). For the test samples used in this study, the robotic system has a maximum capacity of four consecutive dissolution runs per schedule (a schedule is the series of dissolution runs programmed by the operator each time the robot is set up). Several assumptions were made in computing the required times: (a) only one dissolution bath is available for each method; (b) non-concurrent robotic system contains LC system for off-line

**Table 9**
Comparative efficiencies of non-robotic robotic and concurrent sampling robotic dissolution methods. See text for details

| Number of dissolution runs | Activity | Time required (h) | | |
|---|---|---|---|---|
| | | Non-robotic | Robotic | Robot with concurrent |
| 1 | Preparation | 1.0 | 0.5 | 0.5 |
| | Dissolution | 2.2 | 3.0 | 0.3 |
| | Sample assay | 1.2 | 2.3 | — |
| | Total hours | 4.4 | 5.8 | 3.5 |
| 3 | Preparation | 3.0 | 0.5 | 0.5 |
| | Dissolution | 6.6 | 9.0 | 9.0 |
| | Sample assay | 3.6 | 6.8 | — |
| | Total hours | 13.2 | 16.3 | 9.5 |
| | Adj. total* | 39.6 | 16.3 | 9.5 |
| 7 | Preparation | 7.0 | 1.0 | 1.0 |
| | Dissolution | 15.4 | 21.0 | 21.0 |
| | Sample assay | 8.4 | 15.8 | — |
| | Total hours | 30.8 | 37.8 | 22.0 |
| | Adj. total* | 92.4 | 37.8 | 22.0 |

*Adjusted total = total hours $\times \frac{\text{hours per day (24)}}{\text{hours per work day (8)}}$.
Adjustment is for non-robotic dissolution only.

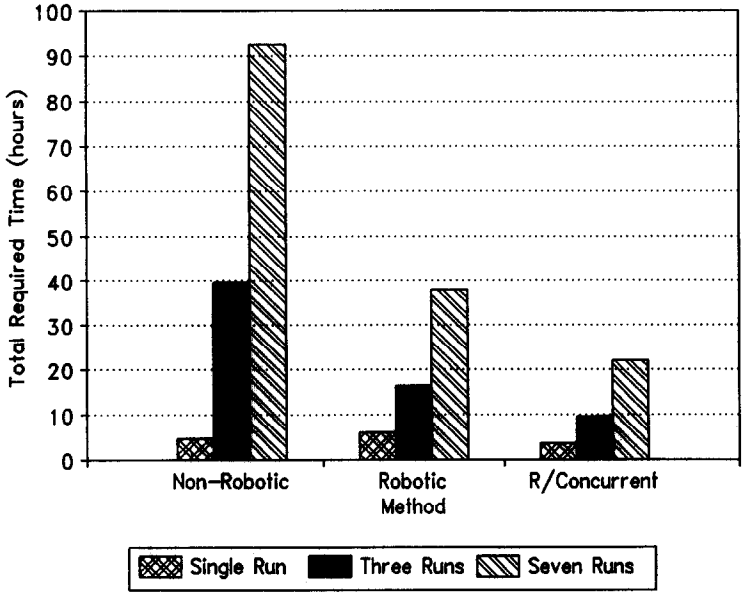


**Figure 6**
Comparison of efficiencies of non-robotic, robotic and concurrent processed robotic dissolution. See text for discussion.

analysis following dissolution run; and (c) sample analysis consists of 26 LC runs of 5 min duration each (six initial standards, 3 × 6 samples and two check standards).

Each dissolution run takes less than 3 h, such that up to three runs can be scheduled and completed during an 8 h work day while four runs can be scheduled on the robot overnight. Computation of the times required for one, three and seven runs were therefore made, with the non-robotic times adjusted for length of the work day. As can be seen from the data, the concurrent robotic operation is the most efficient over several runs and is significantly more productive than the same robot operating in non-concurrent mode. By contrast, the non-robotic method takes almost five times as long to complete seven runs. The pertinent factors influencing the concurrent robotic system's efficiency are the sampling interval and the LC run time. Shorter sampling intervals and longer run times would result in lower efficiency.

Nevertheless, since the robot operates unattended once the runs are scheduled, the analyst is free to engage in other activities. Thus, he or she would still be more productive than without a robotic dissolution system.

## Conclusions

The results of this study verify the accuracy, precision and efficiency of the robotic system. Robotic dissolution data compared well to the non-robotic data. The integrated nature of the concurrent operational mode enables direct adaptation of non-robotic methods without modifications in the dissolution protocol or the analysis conditions. Also, use of a flow injection tube in place of an HPLC column makes direct adaptation of dissolution procedures specifying UV spectrophotometry to the robot possible. Thus, significant productivity gains could result from implementation of this technology.

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