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Solid-state compatibility studies using a high-throughput and automated forced degradation system

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Abstract

As the number of pharmaceutical candidate compounds increases, so does the need for development workflow that is capable of handling more compounds in shorter times. In this paper, the establishment of a high-throughput automated powder compatibility testing system is reported. The integrated robotic system automatically dispenses, weighs, and stores powder samples, and extracts and analyses drug substance using ultra-performance liquid chromatography (UPLC). Although automation of powder testing systems is generally accompanied by difficulties in accuracy and precision, mass tracking at every unit operation allowed the system to be validated. In a standard procedure, drug substance and an excipient were dispensed 1:1 (w/w), stored at 70 °C for 9 days, dissolved in solvents, and analyzed to examine the degradation of drug substance and the increases in related substances. The robot quantitatively discriminate between initial conditions of the incompatible powder mixtures of aspirin and magnesium stearate (Mg-St) prepared with or without the use of a whisk and shaker system, demonstrating the capability for evaluating powder mixtures with varying degrees of homogeneity where the contact area between excipient and drug substance differs. Differential scanning calorimetry (DSC), however, did not clearly distinguish between those powder samples, indicating that DSC is less sensitive to powder conditions. The incompatibility results of aspirin and Mg-St were comparable to those reported previously, demonstrating that the automated testing system is reliable. The robot reduced manual work to one sixth and cut down on the costs of outsourcing. An extensive impact is anticipated on development workflows because this system is applicable not only to compatibility testing but also to analytical method development for drug products.

Keywords: Compatibility; Solid state; High-throughput; Automation; Symyx

1. Introduction

With the advent of new technologies such as combinatorial chemistry and high-throughput screening, an increasing number of candidate compounds are entering the research and development pipelines from drug discovery departments (Sims et al., 2002). Development departments are under growing pressure to optimize the research processes so that scientists can gain knowledge required for formulation selection more efficiently. There is now a need for higher capacity so that candidate compounds can be evaluated while keeping time and development costs at the minimum. Compatibility testing is an essential task that should be performed at the early stage of development as a screening process. Since it usually follows a simple and routine workflow, compatibility testing is amenable to automation and high-throughput techniques that may contribute to the reduction of development time and costs (Carlson et al., 2005). For compatibility studies, differential scanning calorimetry (DSC) (Durig and Fassihi, 1993; Mura et al., 1998a,b; Villalobos-Hernandez and Villafuerte-Robles, 2001; Ceschel et al., 2003; Wyttenbach et al., 2005), isothermal microcalorimetry (IMC) (Peck et al., 1997), scanning electron microscopy (SEM) (Mura et al., 1998b), hot-stage microscopy (HSM) (Mura et al., 1998b) as well as high performance liquid chromatography (HPLC) have been employed for decades. Among these, DSC has widely been used as a rapid method of evaluating any physicochem-

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ical interactions between compounds. The thermal analysis method provides information about potential physical or chemical incompatibilities between formulation components in a very short period of time (usually less than an hour) although the testing results are often not conclusive. In many cases, there is no clear relationship between DSC traces and the compatibilities or incompatibilities between formulation components. HPLC is capable of providing both qualitative and quantitative information on the degradation of drug substances (Mroso et al., 1982). The detailed information is usually conclusive and of great help for formulation selection from a chemical point of view. This separation method, however, requires much longer time and more manual work, resulting in lower-throughput. It is desirable that automation or high-throughput be realized with the advantages of HPLC remaining as they are. As some reports that have appeared in the literature evidence, automation and high-throughput technologies have already been adopted at the development stage (Sims et al., 2002; Fermier et al., 2002).

It is appropriate to prepare and store samples in the solid state when solid-state compatibility studies are performed using liquid chromatography. There are, however, many operational hurdles in dispensing, weighing, and preparing powder samples automatically and reliably. Formerly, therefore, automated high-throughput compatibility testing systems handled liquid samples, but not powder ones, in all the processes, including dispensing, weighing, and storage. Recent improvements in robotics have made it possible to automatically dispense powder samples and weigh vials between unit operations, and an integrated automated high-throughput operation system is commercially available for solid-state compatibility testing (Chandler et al., 2005). In our laboratory, an automated compatibility testing system has been at work for powder mixtures where 48 samples are tested in parallel. The dispensing, weighing, mixing, and storage of powder samples are automatically executed, and ultra-performance liquid chromatography (UPLC) is adopted for separation of degradants and related substances, thus realizing high-throughput generation of experimental results. This paper demonstrates that an essential requirement for successful validation of the analytical system is the implementation of mass tracking at every unit operation and that the analytical system is capable of relevantly controlling the degrees of mixing for powder samples. Some results using aspirin and other in-house compounds are also demonstrated. The extent to which time and costs were reduced by introducing this analytical system and the other advantageous effects of automation and high-throughput technologies on the development procedures are discussed here.

2. Materials and methods

2.1. Materials

Conivaptan hydrochloride amorphous was synthesized in-house. D-Mannitol was supplied by Roquette (Lestrem, France). Microcrystalline cellulose (MCC) was purchased from Asahi Kasei Chemicals (Tokyo, Japan). Magnesium stearate (Mg-St) was supplied by Merck (Whitehouse station, NJ, USA). Titanium oxide (TiO_2) was purchased from Merck (Darnstadt, Germany). Yellow ferric oxide (Fe_2O_3) was supplied by Kishi Kasei (Tokyo, Japan). Acetylsalicylic acid (aspirin), aluminum oxide (Al_2O_3), and light anhydrous silicic acid (SiO_2) were purchased from Kanto chemical (Tokyo, Japan).

2.2. The analytical platform and software

The hardware of the analytical system consists of Powdernium (Symyx Technologies, Santa Clara, CA, USA), Extended Core Module (XCM) (Symyx Technologies, Santa Clara, CA, USA), and a UPLC system (Acquity, Waters Corporation, Milford, CT, USA). Powdernium is a powder-dispensing robot capable of automatically bringing a multi-well metal rack accommodating empty vials onto a balance, carrying a hopper containing drug substance or an excipient over a vial, and letting enough powder fall into each vial so that the predetermined weight is reached. The XCM consists of a three-axis Cartesian robot, pump housing and deck. The XCM stirs, stores, and dissolve the powder mixtures in the vials. The XCM then filters and dilutes the solution to form a test solution. The XCM is equipped with a balance and the robot arm carries a vial onto the balance at every unit operation and then brings it back onto the metal rack. The test solution is analyzed using the UPLC system. All the hardware, operations, and data acquisition are controlled by Symyx Lab Execution software (Symyx Technologies, Santa Clara, CA, USA), which includes Library Studio software and Epoch software. The functions of the software are described below.

2.3. Library design

Library Studio software is used to create a recipe file where samples are displayed in a tabular format (Fig. 1). The recipe

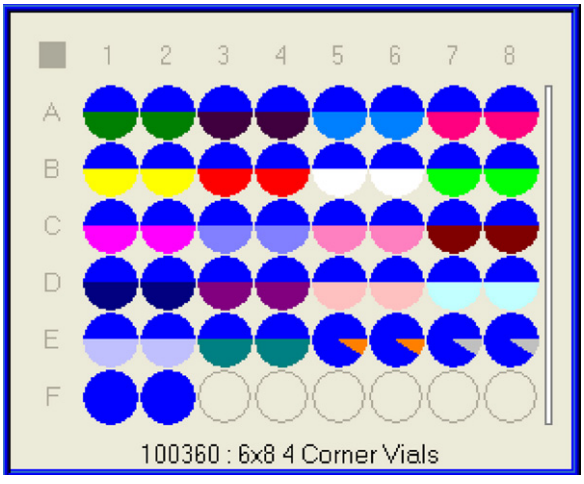

Fig. 1. A recipe file created using Library Studio software is shown. This table describing the composition of powder samples is displayed on the personal computer screen. Forty-eight samples (2 ml vials) are available on a metal rack. The colors in a circle represent chemical components dispensed in a vial containing drug substance. The area of a color part indicates the weight percentage of a chemical component in a vial. In this example, drug substance is represented in blue on the upper parts of the circles in most samples.

file also contains testing parameters such as the number of wells in a rack, vial volume standard (2, 8, or 20 ml), weight of drug substance, weights of excipients, volumes of solvents added, and their dilution factors. The conditions for UPLC analysis, such as injection volumes, run times, and the numbers of injections, were also entered into the recipe file. The solvents used for extracting the drug substance, mobile phases, and final concentrations of drug substance in the test solutions were chosen based on the information acquired at the drug discovery stage.

2.4. Automation of operational procedures

Epoch software controls all operations, including UPLC analysis. All of the operations described below were executed by the robot automatically unless otherwise noted. Powdernium dispenses predetermined weights of a drug substance and an excipient into glass vials previously set on a multi-well metal rack. The metal rack is then manually transferred to the XCM deck. The powder mixtures are then allowed to sit at a constant temperature on the deck. A whisk system and a shaker system are available for stirring the powder mixtures before storage. The initial and validation samples are not stirred or aged, and the drug extraction processes immediately follows. As shown in Fig. 2, the whisk system employs a metal rod that comes down from the top and spins at high speed in a vial, stirring the powder mixtures uniformly one by one. In order to avoid contamination between powder samples, the metal rod is rinsed with solvent, and dried under a nitrogen stream outside the vial. The shaker system is a mechanical installation on the deck that shakes the whole metal rack clockwise (Fig. 2). The whisk and shaker systems work alternately to ensure the mixing uniformity between vials and to control the degree of homogeneity of components in each vial by changing the duration and operation cycles. The powder mixtures were dissolved in solvents, and stirred using the shaker system to facilitate dissolution. Magnetic stirrer bars for the vials are also available on the multi-well rack. The solutions are filtered through a membrane filter, and the filtrates are collected into vials previously set in another metal rack. The solvents are then added to dilute the filtrates and prepare test solutions for chromatographic analysis. Automatic dilution is possible up to three times for an appropriate final concentration of drug substance, and the solutions are stirred at every dilution. Whether powder or solution, sample weights are recorded at every unit operation for subsequent data correction. The metal rack accommodating the test solutions are manually transferred to the UPLC sample holder.

2.5. Validation of the analytical system

Validation of the analytical system was performed using an in-house drug, conivaptan hydrochloride (M.W., 535.05), in coexistence with D-mannitol. Five to fifty milligrams of conivaptan hydrochloride and 20 mg of D-mannitol were dispensed into a 20-ml vial. Fifteen milliliters of a solvent (water/acetonitrile (3/2)) were added to dissolve the powder mixture. The solution was then stirred and filtered through a membrane filter. The filtrate (100 μl) was diluted to 800 μl using the same solvent to achieve a final concentration of 0.04–0.83 mg/ml. A standard solution was prepared using 20 mg of conivaptan hydrochloride without any coexisting excipient according to the experimental procedure stated above. The sample and standard solutions were analyzed using UPLC (Acquity UPLC™ BEH C18, 2.1 mm i.d. × 100 mm, Waters Corporation, Milford, CT, USA). The

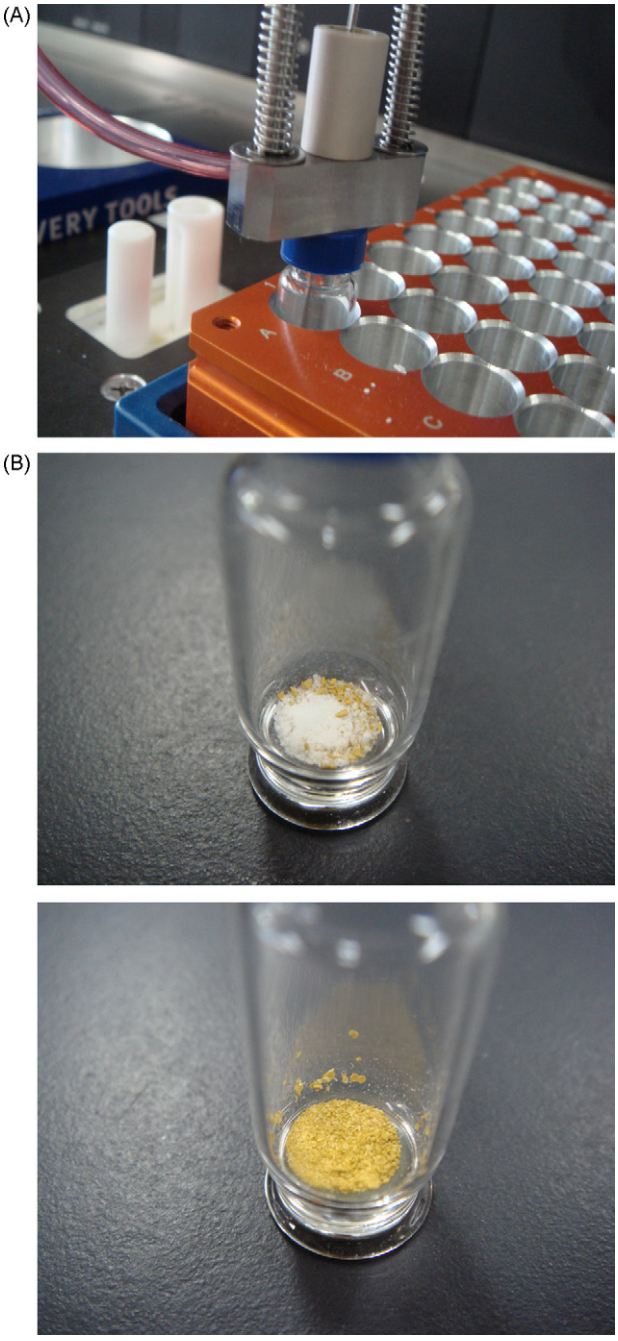

Fig. 2. The stirring systems are shown in photos. (A) The whisk system is shown with a glass vial on a metal rack (orange) in the shaker holder (blue). While the metal rod seen is inserted in the vial, it spins at high speed (whisk system), and the shaker holder rapidly turns clockwise to stir the whole metal rack (shaker system). (B) The effect of the whisk system is visualized using aspirin and yellow ferric oxide. Photos were taken immediately after aspirin (white) and yellow ferric oxide (yellowish brown) were dispensed in a vial (upper), and after the whisk system was employed to stir the powder mixture (lower).

column temperature, flow rate, and mobile phase were 40 °C, 0.5 ml/min, and water/acetonitrile (3/2), respectively. The injection volumes were 5 μl for the analysis of conivaptan.

2.6. The effects of mixing degrees on the interactions between aspirin and Mg-St

Ten milligrams each of aspirin and Mg-St were dispensed into a 2-ml vial. The alternate operation of the whisk and shaker systems or a single bout of vortex mixing by hand was employed to stir the powder mixture. These premixed samples, as well as a powder mixture that did not undergo any mixing process, were stored at 40 °C for 6 days to accelerate the chemical interactions between aspirin and Mg-St. A solvent (1.2 ml, water/acetonitrile (2/3)) was then added to each vial to dissolve the contents. The suspension was stirred and filtered through a membrane filter. Fifty microliters of the filtrate were diluted with an 1150-μl of water/acetonitrile (2/3) to form a sample solution (final conc. approximately 0.35 mg/ml). The solution was analyzed using UPLC (octadecyl silanized silica-based column (Acquity UPLC™ BEH C18, 2.1 mm i.d. × 100 mm, Waters Corporation, Milford, CT, USA)). The column temperature, flow rate, injection volume, and mobile phase were 37 °C, 0.5 ml/min, 3 μl, and acetonitrle/water/acetic acid (60/40/0.1), respectively.

2.7. Compatibility studies of aspirin in coexistence with various excipients

Ten milligrams each of aspirin and one of the seven excipients (aluminum oxide, silicon dioxide, titanium oxide, magnesium stearate, D-mannitol, microcrystalline cellulose, and yellow ferric oxide) were dispensed into a 2-ml vial. Each sample that contained a solid powder mixture was stirred using the whisk and shaker systems and stored at 40, 50 and 60 °C for 6 days to accelerate the interactions between aspirin and the excipient. Preparation of sample solutions and UPLC analysis were performed according to the same procedure stated in Section 2.6. For data analysis, assuming first-order kinetics and that the rate of disappearance of aspirin equals the rate of appearance of degradants, rate constants (k) were obtained by plotting the ln(100 − degradants%) versus time according to Carlson et al. (2005). Arrhenius behavior was assumed to extrapolate and interpolate the rate constants (k) to different temperatures,

$$\ln k = \frac{-E}{RT} + \text{constant}$$

where k is the rate constant at a temperature defined in days^{-1}, E the activation energy (J/mol), R the universal gas constant (J/K mol), and T is the temperature (K).

2.8. Compatibility studies of in-house compounds

Five milligrams of a compound and an appropriate amount of an excipient (0.5, 5, 45, or 50 mg) were dispensed to a 2-ml vial. Each sample was manually vortex mixed and stored at 70 °C for 9 days to accelerate the chemical interactions between the compound and the excipient. A solvent (1, 1.2, or 1.5 ml) was added to each vial to dissolve the contents. The suspension or solution was stirred and filtered through a membrane filter. The filtrate was diluted with a solvent (780–1275 μl) to form a sample solution (final conc. 0.06–1.67 mg/ml). For Drug C, the solution was again diluted to form a final solution. The volume and composition of the solvent used were chosen based on the information obtained at the discovery stage for each compound. The sample solution was analyzed using UPLC. The experimental conditions are summarized in Table 1.

2.9. Differential scanning calorimetry

Powder mixtures used for experiments were divided into four categories. Aspirin and Mg-St (1:1, w/w) were lightly mixed by hand or moderately mixed to homogeneity using the whisk and shaker systems (fresh samples). Some of these powder samples were aged at 40 °C for 6 days (aged samples). One of the powder mixtures was weighed (5–9 mg) and placed in an aluminum pan (4 mm in diameter, max. 45 μl). The sample pan was crimped for effective heat conduction, set inside the furnace of a differential scanning calorimeter (DSC6200, Seiko Instruments, Chiba, Japan), and equilibrated at 40 °C before measurement.

Table 1
Experimental conditions of compatibility testing of candidate compounds

	Drug A	Drug B	Drug C	Drug D	Drug E
Sample preparation					
Target volume of solvent (for dissolution) (ml)	1	1.2	1.2	1.2	1.5
Dilution factor (dilution 1)	6	8.3	16.5	2.5	6.67
Target volume of solvent (for dilution 1) (μl)	1000	880	1080	780	1275
Dilution factor (dilution 2)	–	–	4[a]	–	–
Target volume of solvent (for dilution 2) (μl)	–	–	900[a]	–	–
UPLC analysis					
Injection volume (μl)	5	5	5	3	2
Run time (min)	12	16	30	17	20
Injection number	1	1	1	1	1
Column temperature (°C)	30	25	40	35	40
Flow rate (ml/min)	0.3	0.3	0.3	0.5	0.4

[a] Dilution was performed twice with yellow ferric oxide and red ferric oxide due to the fact that the drug substance in the first diluted solution was too dense to be analyzed.

Table 2
Validation of the analytical system

With mass correction					
Conivaptan Hydrochloride (mg)	5	10	20	30	50
D-Mannitol (mg)	20	20	20	20	20
Average % recovery ($n = 3$)	100.9	100.7	98.2	98.6	98.1
RSD (%)	2.3	1.5	1.3	2.2	1.6
Without mass correction					
Conivaptan hydrochloride (mg)	5	10	20	30	50
D-Mannitol (mg)	20	20	20	20	20
Ave. % recovery ($n = 3$)	108.1	102.5	93.5	98.0	97.3
RSD (%)	4.1	1.9	6.2	3.1	1.6

The sample was then heated at a rate of 10 °C/min to 150 °C. Measurements were made under a constant stream of nitrogen (50 ml/min).

3. Results and discussion

3.1. Validation of the analytical system

In order to examine whether automation of the operations is successful, validation of the analytical system was performed using an in-house drug, conivaptan hydrochloride, by means of addition-recovery experiments (Table 2). The weight of drug substance added ranged from 5 to 50 mg while that of a diluent, D-mannitol, remained at 20 mg. Table 2 (upper) demonstrates that the average percent recovery of conivaptan ranged from 98.2 to 100.9%, which stayed well within the acceptance criterion (100 ± 2%), indicating acceptable accuracy. The maximal value of the relative standard deviations (RSD) was 2.3%, showing good precision. As shown in Fig. 3, linearity was confirmed between 5 and 50 mg (correlation coefficient, 1.00). When an extreme dilution was made with D-mannitol (100 mg D-mannitol added to 5 mg conivaptan hydrochloride), the average percent recovery and RSD were 99.3% and 3.0, respectively (not shown), indicating the excellence of the analytical system.

When validating test methods for drug substances or drug products, it is usually only the operations that occur after the injection of the test solutions into an HPLC system that are investigated. Often, validation procedures do not include the weighing of powder samples, extraction of drug substances, or preparation of test solutions. It should, therefore, be noted that the validation results presented here included all the operational processes, such as the dispensing and weighing of powder samples, extraction of drug substances, injection of test solutions, and chromatographic analysis. When considering automation of an analytical system, the operations conducted before chromatographic analysis are essential, making validation of the chromatographic analysis alone meaningless. Table 2 (lower) lists the values of the average percent recovery and RSD obtained without weight correction. Overall, both recovery and deviation showed unacceptable variations, confirming the vital importance of weighing at every unit operation and of weight correction afterwards.

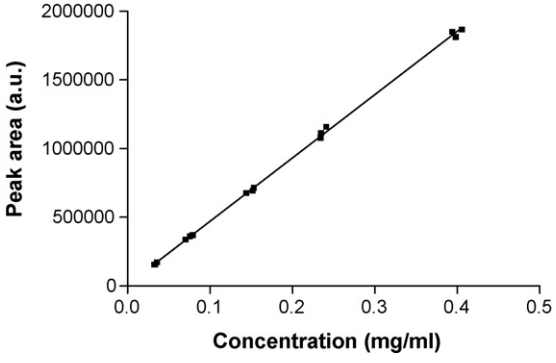

Fig. 3. Linearity is demonstrated (correlation coefficient = 1.00). The horizontal and vertical axes represent the final concentrations of conivaptan hydrochloride in sample solutions, and peak areas measured using UPLC, respectively.

3.2. The effects of the implementation of the whisk and shaker systems

The incompatibility between aspirin and Mg-St has been described in the literature (Mroso et al., 1982; Ceschel et al., 2003; Wyttenbach et al., 2005). Since compatibility testing results should depend on the mixing degrees of drug substance and excipient, it is important to prepare samples of controlled mixing degrees (Wyttenbach et al., 2005). Underestimation would occur if samples were too lightly mixed whereas overestimation would occur if samples were too intensively mixed. The drug substance makes extensive contact with the excipient in an amorphous solid dispersion, whereas a simple mixture would not allow for enough contact between the components, resulting in underestimation. Crystalline components formulated using the direct compaction method allows little contact between the components, whereas a kneaded mixture would allow too much, causing overestimation. Table 3 shows the results obtained using the samples that were dispensed without any subsequent mixing process, moderately mixed by using the whisk and shaker systems, and vigorously mixed by using a vortex mixer by hand before being transferred to the XCM deck. Clearly, degradation depended on the mixing degrees. These results indicate that simple changes in the degree of mixing result in a huge difference for highly incompatible mixtures such as aspirin and Mg-St. The choice of mixing degrees would enable a scientist to evaluate compatibility or incompatibility relevant to intended dosage forms and/or manufacturing processes. The use of the whisk and shaker systems ensures the uniformity between samples to an acceptable degree, considering that they are automatic

Table 3
Compatibility studies of aspirin in coexistence with Mg-St using mixtures of different mixing degrees

	3 days	6 days
No mixing processes	2.9, 2.7 (2.8)	4.4, 4.3 (4.4)
Whisk and shaker systems	7.1, 7.4 (7.2)	9.7, 12.3 (11.0)
Vortex mixer[a]	10.5, 10.1 (10.3)	16.4, 15.9 (16.1)

Results are shown in total amounts (%) of related substances generated after the storage periods. The numbers in parentheses are the averages of the data.
[a] Powder samples were manually vortex mixed.

operations, although the variation was larger than that observed with the simple vortex mixtures (Table 3). It is also important to note that the whisk and shaker systems are capable of regulating the degree of homogeneity of the components in a sample by changing the duration and operation cycles of the mixing systems. Since compatibility testing is usually accompanied by an enormous number of samples, the availability of automated preparation of controlled powder mixtures saves concentrated manual work, which contributes to the avoidance of human errors, securing accuracy, precision, and relevancy of analysis.

3.3. Differential scanning calorimetry

DSC is often employed for compatibility studies to save time. As the effects of mechanical treatment of ibuproxam and picotamide powder samples on DSC traces were reported by Mura et al. (1998a,b), the effects of the whisk and shaker systems on DSC traces of the powder mixtures of aspirin and Mg-St were examined to compare with the chromatographic analyses presented in Section 3.2. Fig. 4 presents the DSC traces obtained using a 1:1 mixture of aspirin and Mg-St. A whisked and shaken sample (mixed sample) and a sample without any particular mixing processes (unmixed sample) were used for measurement. Some of the mixed and unmixed samples were stored at 40 °C for 6 days. Aspirin typically showed an endothermic peak at the melting point (134.8 °C) (Fig. 4A) whereas Mg-St indicated a broader endothermic response centered at 126 °C (Fig. 4B). A minor endothermic deflection was also observed with Mg-St at 95 °C, which is probably due to an impurity, magnesium palmitate, rendering the melting peak of Mg-St broader. As shown in Table 3, after 6 days of storage, the mixed and unmixed samples produced related substances at 11.0 and 4.4%, respectively. Chromatographic analysis demonstrated that a marked difference existed between the mixed and unmixed samples (Table 3). However, when DSC was used, no differences appeared between the fresh samples except a small shoulder observed at 110 °C using a mixed sample (Fig. 4C and D). More importantly, the differences between the DSC traces of the aged samples were not comparable to those observed with chromatography (Fig. 4E and F). Since small endothermic peaks were evident at 110 °C in both traces, aging may have promoted the molecular events that caused the small shoulder observed using the fresh mixed sample to occur. As it did not clearly distinguish between the mixed and unmixed samples, DSC is not always capable of representing the initial conditions of powder mixtures. Although it is a convenient and rapid method, DSC provides an estima-

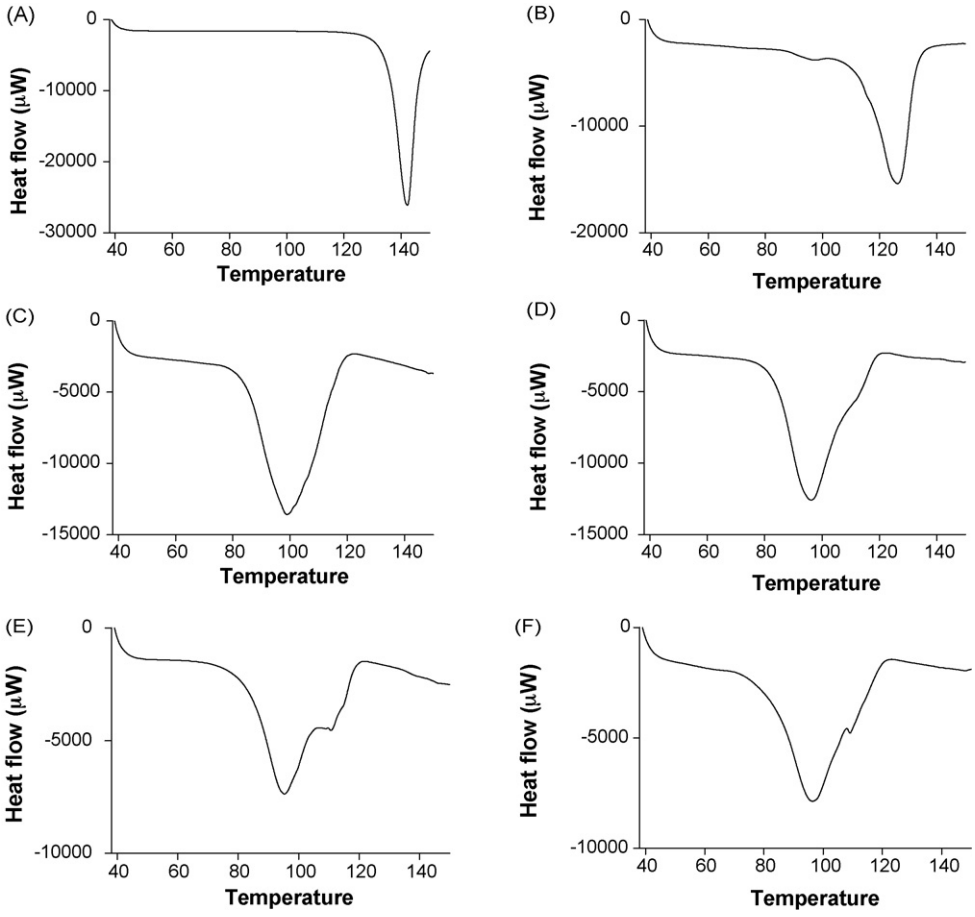

Fig. 4. Compatibility studies of aspirin and Mg-St using differential scanning calorimetry. (A) aspirin, (B) Mg-St, (C) a mixture of aspirin and Mg-St (1:1, w/w) prepared without any particular mixing processes, (D) a mixture of aspirin and Mg-St (1:1, w/w) stirred using the whisk and shaker systems, (E) a mixture of aspirin and Mg-St (1:1, w/w) prepared without any particular mixing processes and stored at 40 °C for 6 days, (F) a mixture of aspirin and Mg-St (1:1, w/w) stirred using the whisk and shaker systems and stored at 40 °C for 6 days.

tion of compatibility or incompatibility between formulation components much more roughly than liquid chromatography. Chromatography data quantitatively reflects the degrees of mixing for powder samples.

3.4. Compatibility studies of aspirin with various excipients using the analytical system

Fig. 5 demonstrates the results of the compatibility testing of aspirin with various excipients carried out using the robot. Samples were stored at 40, 50, and 60 °C. At 50 °C, assay values decreased to a considerable degree using SiO_2 and to a lesser extent using Al_2O_3 and yellow ferric oxide (Fig. 5A). Aspirin was basically compatible with the rest of the excipients. Since a dramatic decrease in assay was observed using Mg-St at 50 °C, Fig. 5A and B shows the time evolution of degradation of aspirin and generation of related substances at 40 °C. As noted earlier, one of the major advantages of using chromatography is that increases in particular related substances can be traced over time. Fig. 5C demonstrates an Arrhenius plot of the degradation kinetics of aspirin in coexistence with Al_2O_3, SiO_2, and Mg-St. By extrapolating the linear lines to 25 °C, the periods after which residual dosages become 95% of the initial values were 84.1 months for Al_2O_3, 3.9 months for SiO_2, and 2.9 months for Mg-St, respectively. Interpolation of the Mg-St line indicates that at 45 °C, it takes 2.2 months to degrade 72% of aspirin, which is rather consistent with a previous finding that it took 2 months to degrade 72% of aspirin in coexistence with Mg-St (1:1) at the same temperature (Ceschel et al., 2003). The 10% difference in time may be explained by the assumption that the whisk and shaker systems used in our system caused a less homogenized sample than theirs. In traditional compatibility studies, the drug substance is usually intensively mixed with the excipient in a mortar (Wyttenbach et al., 2005). The Arrhenius plot indicates that at 35 °C, it takes 3 months to degrade 28% of aspirin, but it took 2 months in their report. This discrepancy may be accounted for by the fact that the assay value at the initial time point was 91% in their report, probably exaggerating the decreases in assay at the later sampling points at lower temperatures. Our system produces testing results comparable to those reported in a previous paper, thus demonstrating the reliability of the automated analytical system.

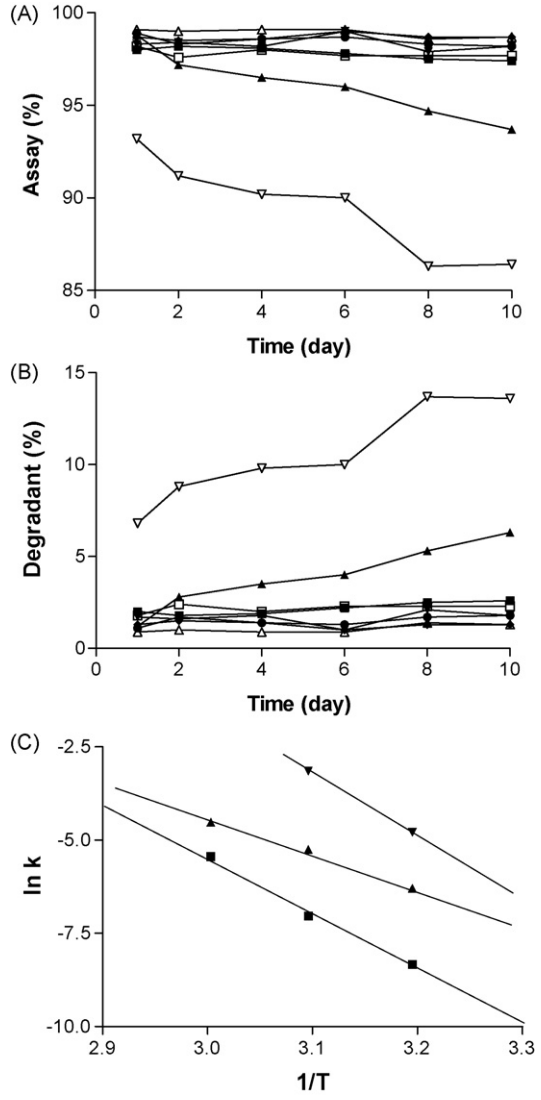

Fig. 5. Compatibility studies of aspirin with various excipients using the robot. (A and B) Aspirin was mixed with an excipient at the ratio of 1:1 (w/w), stirred using the whisk and shaker systems, and stored at 50 °C for 10 days except for Mg-St (stored at 40 °C for 10 days). (A) Assay. (B) Total related substances. Symbols are closed square, Al_2O_3; closed triangle, SiO_2; closed upside-down triangle, TiO_2; closed diamond, D-mannitol; closed circle, MCC; open square, yellow ferric oxide; open upside-down triangle, Mg-St; open triangle, aspirin alone. (C) Arrhenius plots of the degradation kinetics of aspirin in coexistence with Al_2O_3, SiO_2, and Mg-St. Symbols are closed square, Al_2O_3; closed triangle, SiO_2; closed upside-down triangle, Mg-St.

3.5. Other compounds and excipients

In our laboratory, powder compatibility studies are routinely performed using this automated analytical system. Compatibility testing results of five candidate compounds for pharmaceutical use are exemplified in Table 4. Different excipients were used for different compounds due to different expected dosage, dosage forms, and purposes. Powder samples were stored at 70 °C for 9 days. Results are shown as the total amounts of related substances. Drug A is inherently unstable because the original and aged forms contained related substances at 3.7 and 6.4%, respectively. A huge number of related substances (31%) occurred with polyethyleneglycol (PEG) 8000, probably due to the fact that the melting point of PEG8000 is lower than the storage temperature (70 °C). Four to five percent of related substances was observed with celluloses such as croscarmellose sodium, carmellose, carmellose sodium, and microcrystalline cellulose, suggesting that Drug A is generally compatible with celluloses given the initial value (3.7%). Drug B is a stable drug which slightly interacts with microcrystalline cellulose and PEG8000. Drug C is a potent drug substance and its minimal dosage is expected to be very low. Careful selection of excipients was necessary because the ratios of the drug to minor components such as lubricants could approach unity. This compound is incompatible with PEG6000 (26.6%), SLS (44.0%), and sodium sulfite (18.8%). Drug D produced 4.0% related substances in

Table 4
Compatibility testing results of candidate compounds

	Drug A	Drug B	Drug C	Drug D	Drug E
Drug substance					
Drug substance (initial)	3.7	0.1	0.9	N.D.	0.1
Drug substance (70 °C for 9 days)	6.4	0.1	0.8	0.2	0.1
Bulking agent					
D-Mannitol	5.6	0.2	0.8	N.D.	0.1
Maltose	–	–	0.8	–	–
Lactose	5.0	0.1	–	N.D.	0.1
Microcrystalline cellulose	4.6	0.5	0.8	0.7	0.1
Corn starch	5.9	0.1	–	–	–
Dibasic calcium phosphate	9.1	0.1	–	N.D.	–
Calcium silicate	7.6	–	–	–	–
Disintegrant					
Low-substituted hydroxypropylcellulose	5.2	0.1	–	0.4	0.1
Croscarmellose sodium	4.4	0.1	–	N.D.	0.1
Sodium carboxymethyl starch	5.5	0.2	–	N.D.	0.1
Crospovidone	9.8	0.1	–	N.D.	0.1
Carmellose	4.6	–	–	–	–
Carmellose sodium	4.5	–	–	–	–
Partly pregelatinized starch	5.3	0.1	–	–	–
Light anhydrous silicic acid	4.9	–	–	–	–
Binder					
Methylcellulose	–	–	0.8	–	–
Hydroxypropylcellulose	9.1	0.2	–	0.2	0.2
Hydroxypropylmethylcellulose	6.1	0.1	0.8	0.2	0.1
Povidone	10.1	0.1	0.9	0.4	0.2
Lubricant					
Magnesium stearate	16.5	0.1	–	N.D.	0.1
Calcium stearate	5.8	0.3	–	N.D.	0.1
Sodium stearyl fumarate	4.9	–	–	–	–
Coating agent					
Polyethyleneglycol 8000	31.0	0.5	–	4.0	–
Polyethyleneglycol 6000	–	–	26.6	–	1.1
Titanium oxide	5.6	–	–	N.D.	0.2
Talc	5.2	–	–	N.D.	–
Yellow ferric oxide	5.0	–	1.0	N.D.	–
Red ferric oxide	5.5	–	1.0	–	–
Plasticizer					
Triacetine	28.8	–	–	–	–
Surfactant					
Sodium lauryl sulfate	8.8	–	44.0	–	–
Salting-out agent					
Sodium bicarbonate	–	–	0.7	–	–
Sodium sulfite	–	–	18.8	–	–
Sodium citrate	–	–	0.6	–	–
Disodium hydrogen phosphate anhydrous	–	–	0.6	–	–
Monosodium L-glutamate monohydrate	–	–	0.8	–	–
Disodium succinate hexahydrate	–	–	1.0	–	–
Sodium sulfate	–	–	0.8	–	–
Monobasic sodium phosphate	–	–	1.3	–	–
Monobasic sodium citrate	–	–	0.9	–	–
Membrane forming agent					
Ammonio methacrylate copolymer dispersion, type B	–	–	1.0	–	–

In order to minimize time for preparation, powder samples were manually vortex mixed instead of being stirred using the whisk and shaker systems. Results are shown in total amounts (%) of related substances generated after the storage periods.

coexistence with PEG8000, probably due to the melting of the macromolecule, but it is a rather stable drug that slightly reacts with microcrystalline cellulose, low-substituted hydroxypropylcellulose, and povidone. Drug E is also a stable drug that reacts only with PEG6000, and then just slightly. Although these results are shown in total related substances, it is possible that attention is directed to a particular related substance, and when necessary, the pathway and scheme of degradation are discussed on a molecular basis.

3.6. Automated high-throughput screening in solid state

One of the most important factors required for compatibility testing is the capability of handling many samples in a short period of time. DSC is the most rapid method but interpretation of the results is often difficult and it does not always distinguish between powder samples of different mixing degrees. HPLC has been employed as a reliable alternative but it takes a much longer time and higher cost because manual operations are needed for the long experimental procedure including sample preparation, storage, extraction, and data analysis. Pharmaceutical companies worldwide have many candidate compounds in their pipelines, and compatibility studies for all of their compounds are highly expensive. Since it would amount to 40,000 US dollars for one compound if sent out to a domestic commercial research organization, the robot would pay for itself in only a few years. It is desirable that compatibility studies be carried out using the lowest sample mass possible because only a very small amount is usually available for physicochemical characterization at the late discovery or early development stage. Handling an enormous number of a small quantity of samples is a burdensome work that makes operators extremely reluctant and thus would adversely affect the reliability of the results. The automated powder compatibility testing system, which is capable of reliably handling many samples at one time, greatly improves the efficiency of the development processes.

Fig. 6 compares the robot with manual work for compatibility testing using chromatographic analysis. While 18 h of manual work is needed to complete the whole processes, the robot dramatically reduces manual work to 3 h. The robot saves manual work for weighing and preparation of samples for storage as well as chromatographic analysis. Although it takes time to automatically execute all these tasks, the robot can work overnight, thereby compensating for the loss of time. UPLC greatly facilitates analysis and the time needed is one fourth of that for HPLC. An additional 12 h can be saved (from 17 to 5 h) if manual work is available for stirring the powder samples by using a vortex mixer outside the XCM deck instead of the whisk and shaker systems. This option may be used when time is the priority, although automation will be sacrificed, and the degree of mixing may not be as tightly controlled. It is highly advantageous that all the work can be completed in-house because outsourcing would involve various paperwork, including exhibiting research planning, making contracts, and receiving research reports. Priority changes between projects, addition of tested excipients due to formulation and/or manufacturing convenience, and the other major or minor changes can occur at any time during the early stage of development. In-house work can flexibly respond to these sudden changes, avoiding the waste of time and cost. Accumulation of many experimental data in the robot results in the creation of a multiple database, allowing a scientist to find a useful rule on compatibilities and incompatibilities. Since the entire process also works with liquid samples, the robot is applicable to the development of test methods such as the selection of test solutions in assay (combinations of solvents) and in dissolution testing (confirmation of sink conditions, pH dependence of drug solubility), and evaluation of the stability of test solutions. The automated high-throughput analysis system is of extensive use for the whole pharmaceutical development processes.

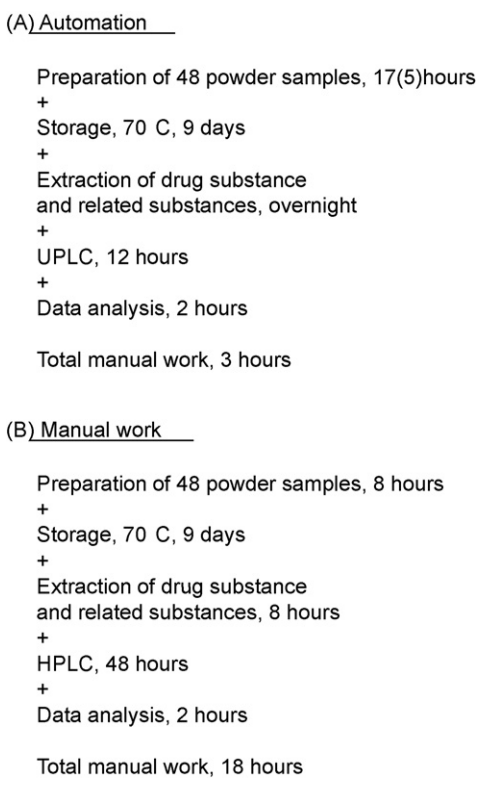

Fig. 6. Comparison of experimental procedures between the robot and manual work. (A) By using the robot, only 3 h are needed for manual work. (B) Without the robot, it takes manual work 18 h to complete all the processes.

4. Conclusion

By using automated unit operations, a high-throughput powder compatibility testing system was established. It was found that mass tracking at every unit operation is crucial for validation. This system is capable of controlling the mixing degrees of powder samples and of evaluating the compatibility or incompatibility relevant to dosage forms and manufacturing processes. The compatibility results using aspirin were comparable to those reported previously, indicating that the automated system is reliable. The use of this system dramatically reduces manual work, saving time and cost. As severe global competitiveness prevails, the establishment of an efficient development framework becomes more and more important in pharmaceutical industry.

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