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In-use testing of extemporaneously prepared suspensions of second generation non-nucleoside reversed transcriptase inhibitors in support of Phase I clinical studies

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Abstract

DPC 961 and 963 are two of a series of prospective second generation non-nucleoside reverse transcriptase inhibitors (NNRTIs) being considered for the treatment of HIV infections. The 'powder in a bottle' approach was used for drug administration for Phase I clinical studies. This new approach consists of compounding the active drug as a suspension or solution at the clinical site immediately before dosing. Prior to clinical use, studies were conducted to determine the compatibility of the drugs with the suspending agent, the recovery of the drugs using the administration procedure and the dissolution profile of the suspensions. The stability of the DPC 961 and 963 in the dosing formulation was followed over a 24-h period using a stability indicating HPLC method. In addition, the dissolution profiles of the suspensions were established for future comparison with solid dosage forms. Although the two drugs have very similar chemical structures, they clearly exhibited different behaviors in liquid/liquid extraction and dissolution experiments. These differences could be related to the physical characteristics of the powders, such as particle size and surface area. The results of the in-use testing of the suspension showed adequate recovery of the drugs from the bottle at two drug levels. The stability of DPC 961 and 963 in the suspending agent was sufficient for constitution and administration of the suspensions at the clinical site.

Keywords: Suspension; HPLC; Dissolution; NNRTI

1. Introduction

DPC 961 and 963 are two of a series of prospective second generation non-nucleoside reverse transcriptase inhibitors (NNRTIs) being considered for the treatment of HIV infections. For Phase I clinical studies, the 'powder in a bottle' (PIB) approach for drug administration is being used. The PIB formulation consists of compounding the active drug as a suspension or solu-

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tion at the clinical site immediately prior to administration to the subject. This new approach is designed to accelerate the entry of new drug candidates into Phase I and is applicable to most orally administered drugs. The liquid formulation also provides optimum drug bioavailability in Phase I studies, while a solid dosage form is being developed for future clinical studies. Although relatively simple from a formulation viewpoint, PIBs present the challenge of having to develop stability indicating methods in complex matrices at a very early stage of development.

The suspending vehicle used in the formulation of DPC 961 and 963 powder in a bottle was a mixture of two commercial solutions: Ora-Sweet® and Ora-Plus®. Both solutions are complex mixtures containing coloring and flavoring agents as well as preservatives. At the clinical site, the drug substances are reconstituted in 15–60 ml of the suspending agent and administered to the subject along with three water rinses.

Before this formulation could be used in the clinic, the compatibility of the drug with the vehicle and the recovery of the drug using the administration procedure (suspension followed by three water rinses) were established. Although analytical methods had been developed for the analysis of DPC 961 and 963 and their respective impurities, these methods had to be modified to eliminate potential interference from components of the vehicle with the determination of degradation products.

Extemporaneously prepared suspensions are commonly used in hospitals for drug administration to patients who experience difficulties in swallowing solid dosage forms or for pediatric patients [1–6]. Previous reports have shown that several commonly used drugs are compatible with the suspending agents Ora-Sweet® and Ora-Plus® [4–6]. However, in these studies, stability was only established by monitoring the loss of potency of the drug. In the present study, the appearance of degradation products during storage of the suspension at room temperature was also monitored, allowing for a more accurate control of drug stability in the suspending agent. For future comparison with solid dosage forms being developed, the dissolution of the suspensions was tested using USP apparatus II.

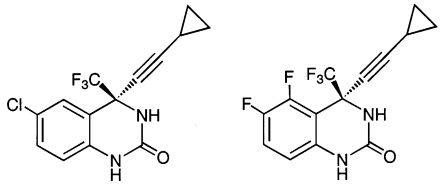

Fig. 1. Chemical structures of DPC 961 and DPC 963.

2. Experimental

2.1. Chemicals

DPC 961, DPC 963 and VS084 were obtained from DuPont Pharmaceuticals Chemical Process Research and Development Department (Chambers Work, NJ). The chemical structures of DPC 961 and 963 are presented in Fig. 1. Methyl paraben was obtained from USP. Ora-Sweet® and Ora-Plus® were from Paddock Laboratories (Minneapolis, MN). All other chemicals were reagent grade or HPLC grade and were obtained from local suppliers.

2.2. Equipment

2.2.1. HPLC system

A Waters HPLC system consisting of a binary pump Model 600, an auto sampler Model 717 and a variable wavelength UV detector model 486 (Waters Corporation, Milford, MA) and a Hewlett Packard HPLC system Model 1050 (Hewlett Packard, Wilmington, DE) were used in this study. The column was a Symmetry C18 150 × 3.9 mm ID, 5 μm (Waters Corp.).

2.2.2. Dissolution equipment

A Distek dissolution system 2100 (Distek Inc., North Brunswick, NJ) equipped with a Instrutex autosampler Model DS500UP (Instrutex International).

2.3. Chromatographic conditions

2.3.1. Isocratic conditions

A mobile phase composed of methanol–water (65:35, v/v) containing 0.1% trifluoroacetic acid (TFA) was used with a flow rate of 1 ml/min and an injection volume of 10 μl.

2.3.2. Gradient conditions

The gradient was linear from 18 to 90% methanol in 25 min. The aqueous phase was composed of potassium phosphate buffer (0.01 M, pH = 7). The flow rate was set at 1.5 ml/min and the injection volume was 50 μl. The sample solvent for standard preparation consisted of methanol–water, (65:35, v/v). The wavelength of detection was 248 nm for DPC 961 and VS084 and 238 nm for DPC 963.

2.4. Sample preparation procedures for suspensions

For liquid–liquid extractions with ethyl acetate or chloroform, the suspension was mixed with 100 ml of solvent for 10 min in a conical flask using a magnetic stir bar and plate. The two layers were allowed to separate and a fraction of the lower organic layer was removed using a transfer pipet. A volume of 5 ml of the organic phase, exactly measured, was evaporated to dryness at ambient temperature with nitrogen and reconstituted with the sample solvent. The solution was injected directly (25 mg strength) or diluted 1:16 with sample solvent and injected (400 mg strength).

2.5. Dissolution experiments

Suspensions were prepared just prior to the start of each experiment by weighing accurately 100 mg of drug substance in a scintillation vial and adding 10 ml of the suspending vehicle. The vial was capped and shaken vigorously by hand for 20 s. The suspension was added to the dissolution vessel through a syringe-cannula assembly to deliver the sample along the wall of the vessel, approximately 5 cm from the bottom. The vial was rinsed once with 10 ml distilled water, which was added to the vessel using the same syringe and cannula. The test was started after complete delivery of the sample. The dissolution medium consisted of sodium dodecyl sulfate 0.3% and 0.063N HCl (pH 1.2). USP apparatus II was used with an initial medium volume of 900 ml, a paddle speed of 50 rpm. The temperature was controlled throughout the experiments at 37 ± 0.5°C. Samples (14 ml) were removed without medium replacement, at 5, 10, 20, 30, 45 and 60 min. After the last pull, the paddle speed was increased to 250 rpm to ensure complete dissolution of the drug and a sample collected at 90 min. All samples were analysed by HPLC using the isocratic method.

2.6. In-use testing of the oral suspension

Suspensions were prepared just prior to the start of each experiment by weighing accurately 25 or 400 mg of the drug substance in cylindrical high density polyethylene (HDPE) bottles. At the 25-mg level, the drug was suspended in 60 ml of suspending vehicle (lowest concentration). At the 400-mg level, the drug was suspended in 15 ml of suspending vehicle (highest concentration).

For the recovery experiments, suspensions were prepared according to the administration procedure to be used in the clinic. Suspensions were prepared in cylindrical HDPE bottles in duplicate at the 25 and 400 mg levels. The suspension was transferred to a conical flask and the bottle rinsed with volumes of water equal to the initial volume of suspending vehicle. To determine the remaining residue, the bottle was finally rinsed with 10 ml of methanol. The residual drug in the methanol rinse was determined using the isocratic HPLC method.

For stability experiments of DPC 961, five bottles of 25-mg suspension and three bottles of the 400-mg suspension were prepared. Two bottles of each strength were assayed for potency and degradation at time zero. One bottle of the 25 mg was assayed at each of the following time points: 1, 8 and 24 h. One bottle of the 400 mg was assayed at 24 h. For DPC 963 stability experiments, three bottles of each strength were prepared as above and tested at time 0 ($n = 2$) and 24 h ($n = 1$). Samples were analyzed using both the isocratic and gradient LC methods. DPC 961 samples were

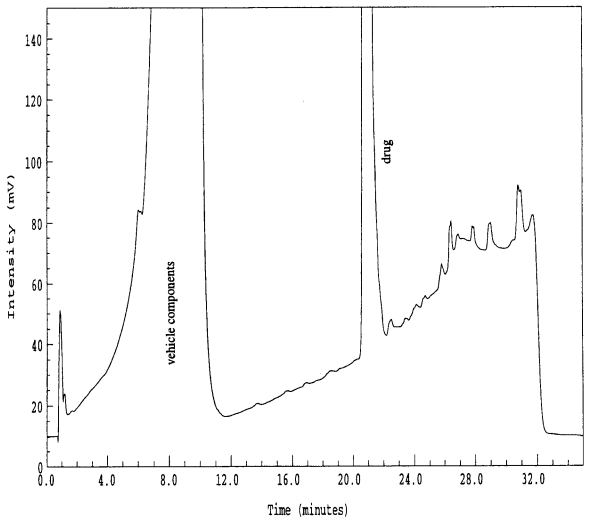

Fig. 2. Separation of DPC 961 from components of the suspending vehicle. Column: Symmetry C18; flow rate: 1.5 ml/min; detection UV 248 nm. Mobile phase A: methanol:water 20:80 v/v. Mobile phase B, methanol:water 80:20 v/v both containing 0.1% TFA. Sample preparation: the suspension (25 mg of DPC 961 in 60 ml of vehicle) was mixed with 100 ml of acetonitrile.

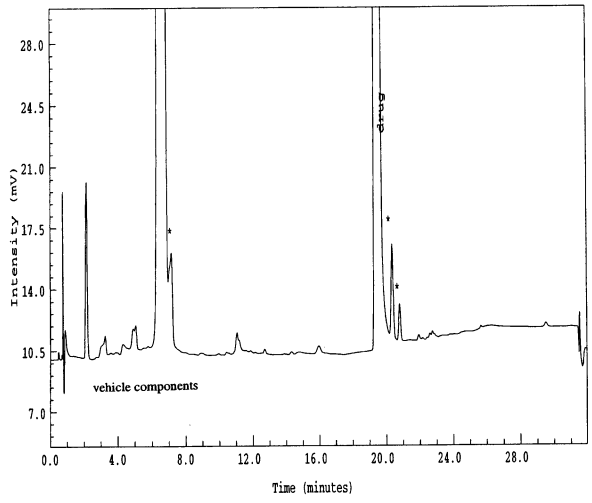

Fig. 3. Chromatogram of an ethyl acetate extract of a DPC 961 suspension. Sample: 25 mg DPC 961 suspended in 60 ml of vehicle. Column: symmetry C18; flow rate: 1.5 ml/min; detection UV 248 nm. Mobile phase A, methanol: phosphate buffer (10 mM, pH 7) 20:80 v/v. Mobile phase B, methanol:phosphate buffer (10 mM, pH 7) 80:20 v/v. *Related substances of DPC 961.

prepared by liquid–liquid extraction with ethyl acetate, while DPC 963 samples were mixed with 50 ml methanol and diluted to a final volume of 200 ml with methanol–water (65:35, v/v).

3. Results and discussion

3.1. HPLC method development for the determination of DPC 961 and DPC 963 and their degradation products

The stability indicating HPLC methods developed for the analysis of the drug substance were evaluated for testing the suspensions. The isocratic method successfully separated DPC 961 and 963 from any excipient peaks and was suitable for potency determination. However, components of both suspending vehicles were found to elute between 6 and 12 min in the gradient method used for determination of degradation products (Fig. 2). Although they did not interfere with the drug itself, they could potentially mask degradation products eluting in that region. One of the interfering peaks was identified as methyl paraben (one of the preservative used in the vehicles), based on retention time. Other interferences were not identified and were thought to be components from the dye or flavoring agent. The modifications to the gradient HPLC method for the determination of degradation products of DPC 961 included a change from a 0.1% TFA (pH = 2)/methanol mobile phase to a phosphate buffer (pH 7)/methanol mobile phase. The change in mobile phase pH did not affect the retention of the active compound, DPC 961 or its synthetic impurities or known degradants as these molecules are not ionized but moved some of the interfering peaks to the solvent front. To further eliminate interfering peaks, a liquid–liquid extraction procedure was also developed for DPC 961 suspensions. The new method eliminated most of the interference from the vehicle. The chromatogram of an ethyl acetate extract of the DPC 961 suspension in Ora-sweet®/Ora-Plus® mixture is presented in Fig. 3. The recovery of DPC 961 at the 25-mg level ranged from 97 to 102% (Table 1). The recovery of SV084, a more polar related substance of DPC

961, at a level of approximately 1 mg was also close to 100%.

When the same analytical method was applied to the analysis of DPC 963 suspensions, the recovery of DPC 963 from extraction with ethyl acetate was found to be highly variable, ranging from 80 to 95% and low compared to DPC 961 as presented in Table 1. Changing the extraction solvent to chloroform did not improve the recovery (Table 1). To investigate further the low recovery of DPC 963 in the liquid–liquid extraction procedure, the extraction with ethyl acetate was monitored over a period of 5 h. Samples of the organic layer (5 ml) were removed after 5, 15 and 30 min, and 1, 2 and 5 h of stirring. The recovery values are presented in Table 2.

Since both DPC 963 and 961 are practically insoluble in water (approximately 10 μg/ml) and in water-based suspending vehicles and highly soluble in the non polar extraction solvents, it was expected that the equilibrium would be reached very quickly. This was not the case with DPC 963, which was completely extracted in ethyl acetate only after 2 h of stirring. A possible explanation for this result is that the viscous vehicle shields the drug from the organic solvent, thus slowing down its transfer to the organic phase.

The final sample preparation for DPC 963 consisted of diluting the suspension first with 50 ml of methanol in a 200-ml volumetric flask and bringing to volume with the methanol/water mixture. This mode of preparation gave acceptable recoveries for DPC 963 even though it seems to introduce a 3–4% bias at the lowest concentration (25-mg level). A representative chromatogram of DPC 963 using the final gradient method is presented in Fig. 4.

3.2. In-use testing of DPC 961 and DPC 963 suspensions

In-use testing was carried out at the lowest and the highest concentrations. The stability of DPC 961 and DPC 963 in the Ora-sweet®/Ora-Plus® mixture was tested over a period of 24 h. The results are reported in Tables 3 and 4 for DPC 961 and 963, respectively. Both suspensions were found to be stable for 24 h.

Table 1
Drug recovery with various sample preparation procedures[a]

Drug name	Level tested (mg)	Methanol/water (%)[b]	Ethyl acetate (%)	Chloroform (%)
DPC 961	25	N/A	97–102[c]	N/A
	400	N/A	99[d]	N/A
DPC 963	25	102	80–95[c]	80–92[c]
	400	100	90[d]	N/A

[a] N/A, condition not tested for this sample.
[b] Total volume of solution was 200 ml.
[c] Range of recoveries.
[d] Mean of two replicate experiments.

Table 2
Recovery of extraction of DPC 963 with ethyl acetate[a]

Sample times	5 min	15 min	30 min	60 min	2 h	5 h
Recovery (%)	83	86	92	97	103	100

[a] Sample DPC 963, 25 mg, in suspension in the suspending vehicle, extracted with ethyl acetate ($n = 1$). Samples (5 ml) of the upper layer were removed at fixed intervals. The change of volume of the ethyl acetate layer was taken into account in the recovery calculations.

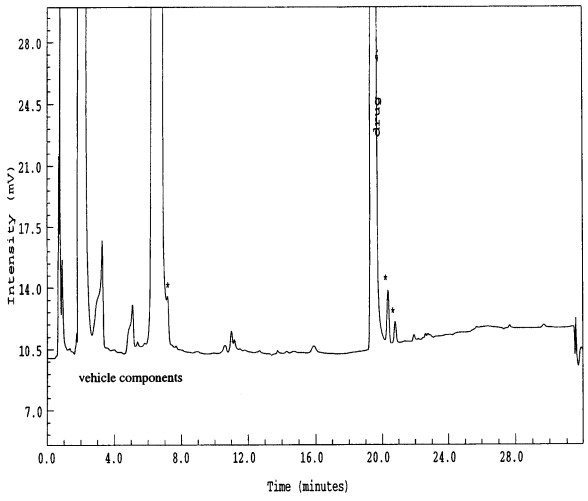

Fig. 4. Chromatogram of DPC 963 suspension. Sample: 25 mg DPC 961 suspended in 60 ml of vehicle. Column: symmetry C18; flow rate: 1.5 ml/min; detection UV 248 nm. Mobile phase A, methanol:phosphate buffer (10 mM, pH 7) 20:80 v/v. Mobile phase B, methanol:phosphate buffer (10 mM, pH 7) 80:20 v/v. Sample preparation: the suspension (25 mg of DPC 961 in 60 ml of vehicle) was mixed with 50 ml of methanol first and with methanol:water 65:35 to a total volume of 200 ml. *Related substances of DPC 963.

The recovery of DPC 961 and 963 after rinsing was determined at the lowest and highest dose levels, using the same rinsing procedure as in the clinic. The results are summarized in Table 5. The residue for DPC 961 was $<1\%$ at the 25-mg strength and $\approx 3\%$ at the 400-mg strength. The residue for DPC 963 was 2% at the 400-mg strength and 5% at the 25-mg strength, indicating that the drug is not completely rinsed from the bottle. However, these recoveries were considered acceptable for use in the clinic.

3.3. Dissolution testing of the suspensions

The dissolution profiles of DPC 961 and 963 suspensions and DPC 961 and 963 drug substance in capsules are presented in Fig. 5. The dissolution of DPC 963 in suspension was close to completion after 60 min, as an additional 30 min at 250 rpm did not increase the percent dissolved significantly. The relatively low final percentage dissolved (83%) was due to incomplete transfer of the suspension into the dissolution vessel. DPC 961 in suspension in Ora-Sweet®/Ora-Plus® mixture, on the other hand, did not dissolve completely even when the paddle speed was increased to 250 rpm. The maximum amount dissolved at 90 min was only 45%. When the neat drug substance in a capsule was tested, the dissolution profile for DPC 963 was comparable to that of the suspension. This was not the case with DPC 961. Suspending DPC 961 seemed to have a favorable effect on dissolution. The difference observed between the two drugs at the end point could be explained by differences in particle size. The lot of DPC 961 used in these experiments had a mean particle size of 20 μm while the lot of DPC 963 had a mean particle size of 6 μm. Based on the solubility of the DPC 961 and 963 in 0.3% SLS (0.6 and 0.4 mg/ml, respectively), dissolution was performed under sink con-

Table 3
Stability of DPC 961 suspensions in Ora-Sweet®/Ora-Plus® mixtures[a]

Time (h)	25 mg in 60 ml		400 mg in 15 ml	
	Potency (%)	Degradation products	Potency (%)	Degradation products
0	98.6[b]	None detected	99.0[b]	None detected
1	102.1[c]	None detected	NA	NA
8	89.8[c,d]	None detected	NA	NA
24	97.6[c]	None detected	98.6[c]	None detected

[a] N/A, condition not tested for this sample.
[b] Mean of two replicates.
[c] $n = 1$.
[d] Low value due to incomplete drug recovery, not to degradation.

Table 4
Stability Of DPC 963 suspensions In Ora-Sweet®/Ora-Plus® mixtures[a]

Time (h)	25 mg in 60 ml		400 mg in 15 ml	
	Potency (%)	Degradation products	Potency (%)	Degradation products
0	102.9[b]	None detected	99.4[b]	None detected
1	NA	NA	NA	NA
8	NA	NA	NA	NA
24	104.0[c]	None detected	100.4[c]	None detected

[a] NA, condition not tested for this sample.
[b] Mean of two replicates.
[c] $n = 1$.

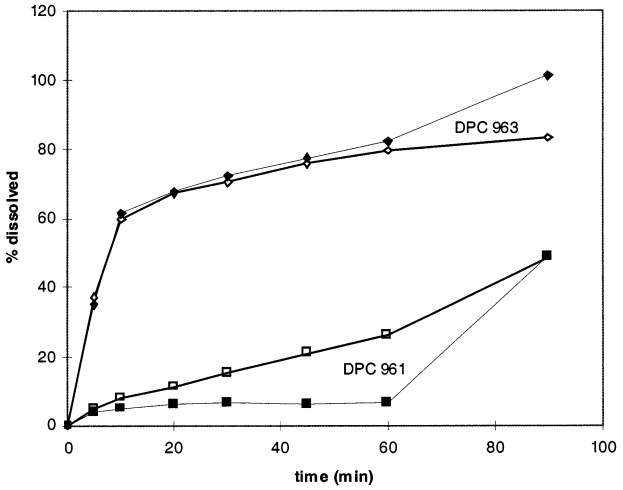

Fig. 5. Dissolution profile of suspensions of DPC 961 and DPC 963. Dissolution medium: 900 ml sodium lauryl sulfate 0.3% and HCl 0.063 N (pH 1.2); USP apparatus II, Paddle speed 50 rpm to 60 min and 250 rpm to 90 min; temperature 37°C, analysis by HPLC (isocratic method). Open symbols: suspension; closed symbols: drug substance in a capsule.

ditions for both drugs and therefore difference in solubility should have no effect on the profile.

4. Conclusion

Interference from suspending vehicle components with the chromatographic determination of DPC 961 and 963 degradation products can be partially overcome by changing the mobile phase pH and/or using a liquid–liquid extraction procedure. The vehicle used in this work was found to slow down the extraction of DPC 963 and to a lesser degree of DPC 961, especially at the lowest concentration. The preliminary data suggested that suspending vehicle improved the dissolution of DPC 961 when compared to neat drug substance in a capsule but had no effect on the dissolution of DPC 963. Both drug suspensions showed sufficient stability to be used at the clinic. DPC 963 was not easily rinsed from the bottle, leaving a residue of 5% at the lowest concentration but the administration procedure was considered acceptable.

The liquid–liquid extraction and dissolution experiments revealed some marked differences in the behavior of DPC 963 and 961. These differences were attributed to differences in physical properties of the two powders. The influence of particle size on dissolution will be investigated further and reported elsewhere.

Table 5
Residual drug in the bottle after completion of the rinsing procedure[a]

	DPC 961 (%)	DPC 963 (%)
25 mg in 60 ml	0.2	5
400 mg in 15 ml	1.5	2

[a] Mean of two replicates. Bottles were rinsed three times with a volume of water equivalent to the volume of suspension.

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