Stability of Extemporaneously Compounded Tacrolimus in Glass Bottles and Plastic Syringes

Tacrolimus is an immunosuppressive agent used to prevent allograph rejection.[1,2] It is available as immediate-release (0.5-, 1-, and 5-mg) or extended-release (0.5-, 1-, 3-, and 5-mg) capsules. The stability of extemporaneous oral suspensions in Ora-Plus/Ora-Sweet and Ora-Plus/syrup has been reported previously.[3-6] The current study was undertaken to evaluate the stability of tacrolimus in Oral Mix vehicle.

Tacrolimus suspensions (0.5 mg/mL) were prepared from bulk powder and from capsules. For bulk powder preparations, tacrolimus USP (117.6 mg; Medisca Pharmaceutique Inc, Montréal, Quebec; lot 48905/A, expiry May 2015; equivalent to 115 mg tacrolimus anhydrous) was wetted with polysorbate 80 (250 µL, Tween 80; Sigma Aldrich, Oakville, Ontario; lot MKBJ0197V) and Oral Mix vehicle (500 µL; Medisca Pharmaceutique Inc; lot I074/A, expiry February 2015) using a mortar and pestle. Oral Mix was then progressively added to achieve the required concentration (0.5 mg/mL, final volume 230 mL). Tacrolimus suspensions from capsules were similarly prepared, without polysorbate 80 (content of 23 × 5-mg Prograf capsules, Astellas Pharma Canada, Inc, Markham, Ontario; lot 044014, expiry April 2015; final volume 230 mL in Oral Mix).

Each preparation was packaged in amber glass bottles (6 × 30-mL fill volume; 50-mL Wheaton type 1 bottles, Medisca Pharmaceutique Inc, lot 24908/A) and amber plastic syringes (48 × 1-mL fill volume; PreciseDose Dispenser, lot 46968/C, with tip cap, lot 55052/A, Medisca Pharmaceutique Inc). Preparations were stored at 5°C or 25°C for up to 90 days. Three bottles were stored at each temperature, and an aliquot was removed from each bottle at each time point for analysis. Half of the syringes were stored at each temperature, and 3 syringes per temperature were used for analysis on each study day.

On each study day, all samples were examined for colour and odour, and pH was measured using a pH 211 meter (Hanna

Table 1. Chemical Stability of Tacrolimus Formulation Prepared from Bulk Powder*

	Mean Concentration ± SD (mg/mL) and Mean % Remaining†			
Study Day	Packaged in Amber Glass Bottles		Packaged in Amber Plastic Syringes	
Storage at 5°C, ambient RH				
Initial (day 0)	0.489 ± 0.012	(100.0)	0.489 ± 0.012	(100.0)
7	0.521 ± 0.015	(106.6)	0.515 ± 0.011	(105.3)
14	0.500 ± 0.017	(102.4)	0.503 ± 0.004	(102.9)
30	0.485 ± 0.008	(99.2)	0.489 ± 0.007	(100.1)
45	0.510 ± 0.008	(104.2)	0.516 ± 0.020	(105.5)
60	0.533 ± 0.010	(108.9)	0.518 ± 0.014	(106.0)
75	0.505 ± 0.004	(103.2)	0.504 ± 0.011	(103.0)
90	0.494 ± 0.005	(101.1)	0.484 ± 0.007	(99.0)
Storage at 25°C, 60% RH				
Initial (day 0)	0.489 ± 0.012	(100.0)	0.489 ± 0.012	(100.0)
7	0.512 ± 0.007	(104.7)	0.518 ± 0.007	(105.9)
14	0.518 ± 0.022	(105.9)	0.496 ± 0.028	(101.4)
30	0.494 ± 0.014	(101.1)	0.498 ± 0.005	(101.8)
45	0.511 ± 0.011	(104.6)	0.511 ± 0.010	(104.6)
60	0.533 ± 0.016	(109.0)	0.513 ± 0.010	(105.0)
75	0.492 ± 0.014	(100.6)	0.494 ± 0.008	(101.0)
90	0.488 ± 0.010	(99.8)	0.469 ± 0.008	(95.9)

RH = relative humidity, SD = standard deviation.
*Nominal concentration: 0.50 mg/mL.
†Mean concentrations are based on 3 separate samples; the percentage remaining is relative to the initial concentration measured on day 0.

Instruments, Montréal, Quebec). Tacrolimus concentration was then assayed by high-performance liquid chromatography with ultraviolet detection (HPLC-UV).

For the HPLC-UV analysis, each sample (100 µL) was first diluted with acetonitrile (400 µL, HPLC-grade, Fisher Scientific, Ottawa, Ontario; lot 141693), vigorously vortexed (20 s, Vortex Genie 2, Fisher Scientific), and centrifuged (13 000 rpm for 10 min; model 5424 centrifuge, Eppendorf Canada, Mississauga, Ontario). The mixture separated into a lower phase (12% v/v) and an upper phase (88% v/v). An aliquot of the supernatant (150 µL) was then diluted with purified water (150 µL; Milli-Q Synthesis A10 system, Millipore, Etobicoke, Ontario) and analyzed by HPLC-UV.

The HPLC system (Prominence UFLC, Shimadzu, Laval, Quebec) consisted of a degasser, a binary pump (0.9 mL/min), a photodiode array detector (210 nm), a refrigerated autosampler (5°C), a column oven (50°C), and a Kinetex XB C18 column (4.6 × 100 mm, 5 µm, Phenomenex, Torrance, California; lot 5705-26). The mobile phase consisted of acetonitrile and water (30:70).

Tacrolimus standards (0.6, 0.5, 0.4, 0.3, and 0.2 mg/mL) were prepared in Oral Mix vehicle for validation of the method. Injection samples were prepared from these standard samples as described above and immediately analyzed by HPLC-UV (triplicate preparations, triplicate injection samples, all repeated on days 0, 3, and 7). The tacrolimus peak eluted at about 3.8 min. The coefficient of regression (r^2) of the calibration curve was at least 0.998. The intraday coefficient of variation calculated for triplicate injection samples was not more than 0.6% in all cases. The intraday coefficient of variation calculated between sample preparations at the target concentration was not more than 3.3%. Similarly, the interday coefficient of variation at the target concentration was 3.1%.

A stress degradation study was performed using 1:1 mixtures of the 0.5 mg/mL standard sample with water, hydrochloric acid (HCl) 0.1 mol/L, sodium hydroxide (NaOH) 0.1 mol/L, and hydrogen peroxide (H_2O_2) 3% (incubated at 60°C for 4 h). Samples for HPLC injection were prepared as described above. Degradation of tacrolimus was observed for all conditions (recovery 58.7% in water, 89.6% in HCl, 41.0% in NaOH, and 64.1% in H_2O_2). No peak overlap or signal interactions with excipients or with degradation products were observed.

On each study day, all suspensions were easily resuspended, no changes in colour or odour were noticed, and the pH remained constant at 4.4. The mean tacrolimus concentration was not less than 90.0% of initial concentration for each tested condition (Tables 1 and 2). One measured value was 111.8% after 60 days of storage, but it is believed that analytical error was responsible for this high value, as it was not repeated on days 75 and 90 (Table 2).

All tacrolimus suspensions (0.5 mg/mL in Oral Mix, prepared from capsules or bulk powder, stored in glass bottles or plastic syringes at 5°C or 25°C) were stable for at least 90 days. These results compare favourably with those for a previously reported tacrolimus suspension in a mixture of Ora-Plus and simple syrup, which was stable for at least 56 days.[6]

Table 2. Chemical Stability of Tacrolimus Formulation Prepared from Tablets*

	Mean Concentration ± SD (mg/mL) and Mean % Remaining†			
Study Day	**Packaged in Amber Glass Bottles**		**Packaged in Amber Plastic Syringes**	
Storage at 5°C, ambient RH				
Initial (day 0)	0.490 ± 0.015	(100.0)	0.490 ± 0.015	(100.0)
7	0.491 ± 0.029	(100.2)	0.481 ± 0.050	(98.2)
14	0.471 ± 0.028	(96.2)	0.452 ± 0.013	(92.2)
30	0.478 ± 0.042	(97.5)	0.488 ± 0.039	(99.6)
45	0.537 ± 0.011	(109.5)	0.514 ± 0.012	(105.0)
60	0.535 ± 0.023	(109.2)	0.515 ± 0.012	(105.1)
75	0.528 ± 0.012	(107.7)	0.499 ± 0.005	(101.9)
90	0.509 ± 0.007	(103.9)	0.488 ± 0.020	(99.6)
Storage at 25°C, 60% RH				
Initial (day 0)	0.490 ± 0.015	(100.0)	0.490 ± 0.015	(100.0)
7	0.510 ± 0.011	(104.1)	0.493 ± 0.012	(100.6)
14	0.496 ± 0.010	(101.2)	0.458 ± 0.015	(93.5)
30	0.512 ± 0.020	(104.4)	0.504 ± 0.018	(102.9)
45	0.521 ± 0.019	(106.4)	0.500 ± 0.005	(102.1)
60	0.548 ± 0.014	(111.8)	0.505 ± 0.018	(103.1)
75	0.511 ± 0.011	(104.3)	0.500 ± 0.011	(102.0)
90	0.502 ± 0.018	(102.5)	0.475 ± 0.011	(96.9)

RH = relative humidity, SD = standard deviation.
*Nominal concentration: 0.50 mg/mL.
†Mean concentrations are based on 3 separate samples; the percentage remaining is relative to the initial concentration measured on day 0.

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