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A New Formulation of Controlled Release Amitriptyline Pellets and Its *In Vivo/In Vitro* Assessments

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Controlled-release amitriptyline pellets (ATP) were formulated and its oral bioavailability was assessed in human volunteers after oral administration under fasting conditions. Core pellets were prepared using a CF granulator by two different methods (powder layering and solvent spraying) and coated with Eudragit RS or RL 100. Physical characteristics and dissolution rates of core pellets and coated pellets were evaluated to optimize the formulation. Powder layering method resulted in a better surface morphology than solvent spraying method. However, physical properties of the products were poorer when prepared by powder layering method with respect to hardness, friability and density. The dissolution profile of amitriptyline coated with Eudragit RS 100 was comparable to that of commercially available amitriptyline enteric-coated pellets (Saroten® retard). After the oral administration of both products at the dose of 50 mg, the mean maximum concentrations (C_{max}) were 36.4 and 29.7 ng/mL, and the mean areas under the concentration-time curve (AUC_{0-96}) were 1180.2 and 1010.7 ng.h/mL for ATP and Saroten retard, respectively. The time to reach the maximum concentrations (T_{max}) was 6 h for both formulations. Statistical evaluation suggested that ATP was bioequivalent to Saroten retard.

Key words: Amitriptyline, Pellets, Enteric-coating, Dissolution, Bioavailability

INTRODUCTION

Amitriptyline (AT) is one of the most commonly prescribed tricyclic antidepressants that inhibit monoamine reuptake. Clinical studies have demonstrated that sustained-release AT treatment is more clinically useful to initiate and maintain the anticholinergic properties compared to immediate release or conventional therapy (Pickup *et al.*, 1982; Debbas *et al.*, 1989).

The use of coated pellets, granules, or microparticles is one of the numerous advances in formulation of sustained-release drugs (Ghebre-Sellassi, 1994; Bechgaard *et al.*, 1982). They allow decreased frequency of dosing and thus minimize the side effects of the drug and ensure good dispersion of the drug throughout the gastrointestinal tract, avoiding local irritation of the gastric mucosa. Currently marketed controlled-release product of AT, Saroten retard (Lundbeck, Denmark) containing 50 mg of AT, may be satisfactory in exerting pharmacologic effects. Saroten® retard contains a mixture of fast- and sustained-released pellets at the same mixing ratio. Therefore, it needs to prepare the sustained- and fast-released pellets separately. However, the mass production of the marketed product is somewhat time-consuming and costly. This drawback can be overcome by designing a suitable AT controlled-release pellets. The formulation involves the preparation steps using biocompatible polymers such as Eudragit which is more flexible and suitable for coating of pellets (McGinity, 1989; Cole *et al.*, 1995), and may overcome these problems and even provide benefits. However, it is essential that the generic preparations have the same bioavailability characteristics as the innovator preparation before they can be safely replaced the innovator in treating patients. To establish bioequivalence of generic products, a single-dose, fasting, two-way crossover bioavailability study using healthy human volunteers is generally accepted as an established method for evaluating bioequivalence (Yuen *et al.*, 1999; Peh *et al.*, 1999).

The aims of the present study were to develop a new

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controlled-release AT pellets (ATP) and subsequently to compare the bioavailability with the market product, Saroten® retard.

MATERIALS AND METHODS

Materials

Amitriptyline HCl was obtained from Plantex (Netanya, Israel) and desipramine was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Lactose 200 mesh and Avicel PH-101 were obtained from DMV (Veghel, Holland), dicalcium phosphate and talc were obtained from Hwail Pharma Co. (Hwasung, Korea) and Su Sung Pharma Co. (Ansan, Korea), respectively. Nonpareil seed was obtained from Freund (Tokyo, Japan) and Eudragit RS 100 and Eudragit RL 100 were purchased from Röhm Pharma (Darmstadt, Germany). All chemicals used were of analytical grade.

Preparation of ATP

All the binders except for gelatin were dissolved in ethanol immediately before use (4% w/w). Air bubbles, formed during the mixing process, were driven out by sonication. Gelatin was first hydrated in cold water overnight and then, dissolved by heating the mixture to 50°C. The temperature of the solution was maintained at room temperature during the pelletization process.

Two different methods were used to prepare the core pellets. The one was the powder layering method, and the other was the solvent spraying method.

In the powder layering method, nonpareil seeds (32-42 mesh) were placed in the granulation chamber of CF-granulator (Keum Sung KCC-360, Seoul, Korea) and allowed to tumble for 5 minutes prior to addition of the binder solution. The binder solution was sprayed until the seeds became moist. After spraying was stopped, the powder (AT and excipients) was fed, and the particles were allowed to tumble for 3 minutes. The binder solution was sprayed again and the whole process was repeated. The solution was sprayed until the required amount of powder was fed, then the wet pellets were dried for 5 minutes using the fluid-bed drier. The pellets were further dried in oven at 45°C for 4 h before sieving and coating. The formulation used for the preparation of amitriptyline core pellets by the powder layering method is presented in Table I.

In the solvent spraying method, the binder and AT were dissolved in solvent and the mixture was used as the binder solution. The binder solution was sprayed while the mixed powder was fed continuously. When the solution was exhausted, the spraying and powder feeding were terminated. The pellets were partially dried in the same chamber using the fluidizing air. After 5 minutes, the particles were transferred to a tray and further dried in an oven at 45°C for 4 h. The dried pellets were sieved, weighed and stored in vinyl bags. The formulation employed in the solvent spraying method is given in Table II.

Table II. Formulation used for the preparation of amitriptyline core pellets by solvent spraying method

Ingredients	Formulation			
	SS-1	SS-2	SS-3	SS-4
Amitriptyline HCl	1,000	1,000	1,000	1,000
Avicel PH-101	1,700	–	1,700	–
Lactose	–	1,700	–	1,700
Silicon dioxide	30	30	30	30
Eudragit RL 100	4%	4%	–	–
Eudragit RS 100	–	–	4%	4%
Solvent	Ethanol	Ethanol	Ethanol	Ethanol
Nonpareil seed	400	400	400	400

Table I. Formulation used for the preparation of amitriptyline core pellets by powder layering method

Ingredients	Formulation						
	PL-1	PL-2	PL-3	PL-4	PL-5	PL-6	PL-7
Amitriptyline HCl	1,000	1,000	1,000	1,000	1,000	1,000	1,000
Avicel PH-101	1,700	–	–	1,700	1,700	–	–
Lactose	–	1,700	–	–	–	1,700	–
Dicalcium phosphate	–	-	1,700	–	–	-	1,700
Talc	30	30	30	30	30	30	30
Gelatin	–	–	–	2%	–	–	–
Eudragit RL 100	4%	4%	4%	–	–	–	–
Eudragit RS 100	–	–	–	–	4%	4%	4%
Solvent	Ethanol	Ethanol	Ethanol	Water	Ethanol	Ethanol	Ethanol
Nonpareil seed	400	400	400	400	400	400	400

Table III. Composition of coating solution for controlled-release amitriptyline pellets

Ingredients	Formulation			
	1	2	3	4
Eudragit RS 100	100	100	100	100
Eudragit RL 100	–	–	–	10
PEG 6000	10	–	–	–
Dibutylphtalate	–	10	–	10
Triethylcitrate	–	–	10	–
Talc	50	50	50	50
Ethanol	1820	1820	1820	1820

The core pellets were coated using a CF-granulator. Coating solutions containing Eudragit RL 100 or Eudragit RS 100 were prepared at 4% w/w solids and were applied in bed temperatures of 43-45°C. After film coating was carried out, the pellets were dried at 40°C for 24 h prior to additional testing. Pellets containing different diluents and plasticizers were prepared for the desired drug release pattern of AT. The composition of coating solution is presented in Table III.

Sieve analysis

The size distribution of the pellets was evaluated by the sieve analysis technique using 14-20 mesh screens. The sieve nest was shaken until no further change in weight distribution of particles was observed with continued shaking.

Hardness

The hardness of the pellets was measured using a balance petri-dishes and water. A pellet was placed in the cap center of the petri dish on the balance, and tared. Then the petri dish was placed on the pellet and water was added to petri dish until the pellet was crashed. The weight when the pellet was crashed was regarded as the hardness of the pellet.

Bulk density

The bulk densities of pellets were measured using an ABD Fine Particle Characteristics Measuring Instrument (Model ABD-72). After the sample container (100 mL) was placed on the measuring round plate, the supply switch was turned on. When the sample container became full, the supply switch was turned off, and the heap portion was slashed off with the spatula. The sample sticking around the container was brushed off and the weight was measured. The weight of the empty container was weighed in advance and was subtracted from total weight (sample weight plus container weight). The bulk densities were determined from the weight and volume of the particles.

Friability

Exactly fifty grams of pellets were placed in a Friabilator (Whunwon, Model 4SJ10GA-A) and rotated for 4 minutes at 25 rpm. The pellets were then screened to remove fine powders formed, reweighed. Friability of pellets was expressed as the percentage of the weight which was lost during rotation.

Scanning electron microscopy

The morphology of both core and coated pellets was examined by scanning electron microscopy (SEM, Jeol JSM-35CF, Tokyo, Japan). Samples were freeze dried, cross-sectioned and then placed onto aluminum stubs coated with adhesive. The cross-sections of the pellets were coated with gold under vacuum and examined under the microscope to visualize the surface characteristics of the pellets.

Dissolution studies

The dissolution characteristics of AT from pellets coated with 4% Eudragit RS 100 or RL 100 were studied using a modified USP XXIII type 2 apparatus (Basket method) in the simulated gastric fluid (pH 1.2) for 1 h followed by pH gradient with phosphate buffer solution (pH 9.3) thereafter in order to simulate gastrointestinal conditions. A sufficient amount of ATP was agitated at 100 rpm in a dissolution vessel containing 500 mL of pH 1.2 buffer solution at 37±0.5°C. The dissolution medium (20 mL) was collected after 1 h and replaced the equal volume of fresh dissolution medium (pH 1.2) with additional 200 mL of pH 9.3 phosphate buffer to adjust pH 5.0. After one hour, 20 mL of the medium was again withdrawn and replenished the same volume in addition to 200 mL of phosphate buffer made up to 900 mL medium (pH 6.8). At 3 h and beyond, same volumes of the sample were collected at 1 h interval until 8 h without replacement of medium. The concentrations of AT in the collected dissolution medium were determined by HPLC.

Bioavailability studies

In an open-label, two-way crossover design, the bioequivalence of ATP, containing 50 mg AT, in reference to Saroten® retard (Batch No. F246; Lundbeck Kopenhagen, Denmark) was evaluated in overnight-fasted 4 healthy male volunteers (age 22 to 35 and weight 62 to 78 kg) following oral administration. Informed consent was obtained from all volunteers who were medication free for at least 3 days prior to the study. A minimum of one week wash out period was allowed between each dosing period. Plasma was obtained at predetermined time intervals, and the concentration of AT was determined by HPLC. The area under the concentration-time curves (AUCs) were calculated using the trapezoidal method. The differences

between AUCs of both treatments were calculated for statistical significance using the Student's *t*-test. The mean maximum concentrations (C_{max}) and the time to reach the maximum concentration (T_{max}) were directly read from the plasma concentration profiles.

HPLC determination of AT plasma levels

AT levels in plasma were determined using a validated HPLC method (Ghahramani and Lennard, 1996; Milada, 1992; Rolf and Harald, 1986). A portion (0.1 mL) of the desipramine solution (1.0 ng/mL, internal standard) was added to 1.0 mL of plasma. For extraction, 0.1 mL of 5.0 M sodium hydroxide and 5.0 mL of butan-1-ol in *n*-hexane (2% v/v) were added to the plasma sample, and the mixture was vortexed for 10 minutes. Following centrifugation (2,000 rpm, 4°C for 5 min), the organic phase was transferred into a clean test tube. The organic phase was dried under gentle nitrogen stream. The residue was reconstituted with 300 μL of the mobile phase, and 100 μL was injected onto the column. The HPLC system consisted of an isocratic pump (Hitachi L-7100, Ibaraki-ken, Japan), an autosampler (Hitachi 7200), an UV detector (Hitachi L-7400) and an integrator (Hitachi D-7500). The column used was a Luna C_{18} column (5 μm, 25 cm×4.6 mm I.D.; Phenomenex, Torrance, CA, USA). The mobile phase was a mixture of acetonitrile/water containing 1% v/v triethylamine (TEA), adjusted to pH 3.0 with orthophosphoric acid (36: 67, v/v), and the flow rate was 2.0 ml/min. The detection wavelength was 240 nm.

Statistics

The statistical differences were estimated with Student's *t*-test ($p<0.05$).

RESULTS AND DISCUSSION

Physical properties of pellets were evaluated and the results are presented in Table IV. The 14-20 mesh size pellets were selected for the subsequent coating process because pellets of this size are easy to handle. The powder layering method showed a better size distribution than the solvent spraying method. In the powder layering method, more than 90% of pellets were sized in 14-20 mesh (except PL-4). In this study, friability tests provided good comparative values (0.10-0.29%). A weight loss of less than 0.8% is generally considered satisfactory for tablets. For pellets, however, the acceptable value could be higher to compensate for the higher surface area per unit weight and the subsequent frictional forces involved. Bulk density of pellets is indicative of the packing properties of particles and is greatly influenced by the diameter of pellets. In our study, bulk density should be over 0.6 g/mL to fill 230 mg pellets (50 mg as amitriptyline) in #2 capsule (volume 0.38 mL). The minimum bulk density of tested pellets was higher than 0.7 g/mL, thus all the pellets prepared can be filled easily into #2 capsule.

Microscopic characterization of ATP

ATP was prepared by both the powder layering and the solvent spraying techniques. In the solvent spraying method, AT was homogeneously mixed with the binder solution. The scanning electron micrographs in Fig. 1 reveal good spherical shapes of ATP prepared by the powder layering method and less regular shape by the solvent spraying method.

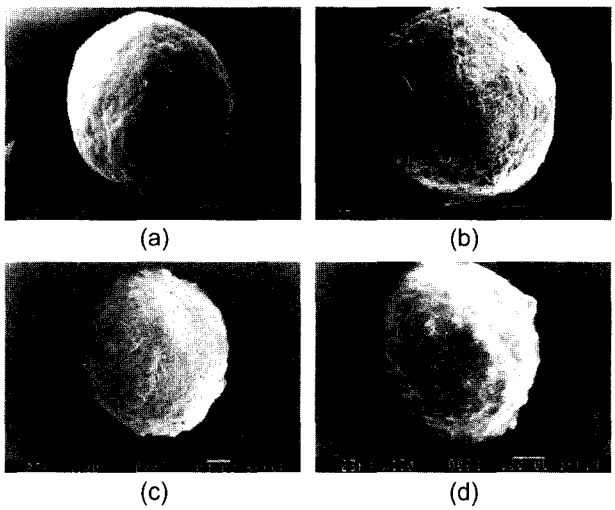

Fig. 1. Scanning electron photomicrographs of ATP (A) core pellet prepared by powder layering method, (B) coated pellet prepared by powder layering method, (C) core pellet prepared by solvent spraying method, and (D) coated pellet prepared by solvent spraying method.

Table IV. Physical properties of the pellets

Formulation	Powder layering method					Solvent spraying method			
	PL-1	PL-4	PL-5	PL-6	PL-7	SS-1	SS-2	SS-3	SS-4
% of 14-20 mesh	93.5	65.0	94.2	95.1	94.3	66.2	58.3	65.3	59.3
Hardness (g)	*129 ± 21	135 ± 3	130 ± 13	151 ± 6	199 ± 8	231 ± 26	240 ± 20	240 ± 16	261 ± 16
Bulk density (g/mL)	0.78	0.80	0.77	0.80	0.94	0.82	0.83	0.82	0.82
Friability (%)	0.29	0.18	0.20	0.13	0.06	0.15	0.18	0.17	0.10

*mean±S.D.(n=3)

Dissolution studies

To examine the potential use of Eudragit RS 100 and RL 100 for a controlled-release formulation of AT, *in vitro* release studies in gastrointestinal simulated fluids were performed using the pH variation method. The release profile obtained from ATP made with Eudragit RS 100 with a gradient of pH was slower than that from ATP made with RL 100. The release patterns obtained from both formulations followed a pseudo zero-order kinetics for about 4 h, and then deviated to form another near linear release profile which is of a slower rate than the initial release. In this study, computational simulations were performed using SCIENTIST® (MicroMath Scientific Software, Salt Lake City, UT, USA) to determine the optimum dissolution rate of the new controlled-release formulation with made-to-order release characteristics for ATP. The intrinsic absorption rate constant, k_a, was systematically varied from 0.1 to 3 h^{-1}, and the optimum absorption rate was found to be $k_a = 0.2\ h^{-1}$ for the controlled-release AT providing more than 80% drug release maintained over the period of 24 h. Eudragit RS 100, in this study, showed a sustained release pattern similar to the target profile.

Among ATP formulations incorporated with various diluents, those prepared with lactose and Avicel PH-101 showed a satisfactory retard release pattern. ATP containing Avicel showed a fast dissolution rate, while the dissolution profile of ATP employing dicalcium phosphate was affected by pH of dissolution medium (Fig. 2). As observed from the data, lactose showed a similar drug release pattern to the target profile. The effect of plasticizer in the coating solution was also investigated. The ATP coated with dibutylphthalate (DBP) and triethylcitrate (TEC) showed faster release patterns than that containing polyethyleneglycol 6000 (PEG 6000). As shown in Fig. 3, the formulation incorporated with PEG 6000 showed the most similar release pattern to the simulated one. Thus, the controlled-release formulation of ATP was prepared with lactose as

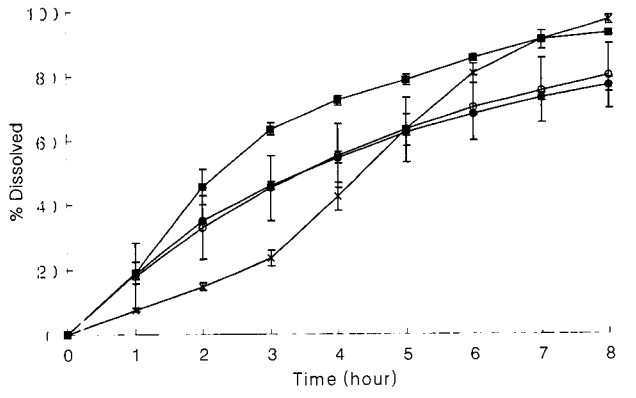

Fig. 2 Dissolution profiles of amitriptyline from coated pellets containing different kinds of diluents. Keys; ○: Simulation, ●: Lactose, ■: Avicel PH-101, ×: Dicalcium phosphate.

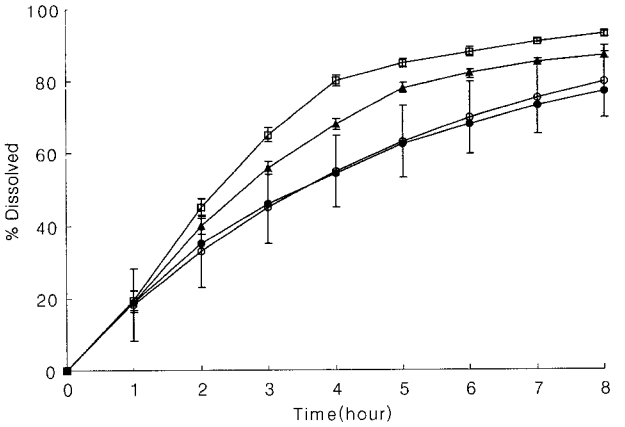

Fig. 3. Influence of plasticizers incorporated in coating solution on the dissolution of amitriptyline from coated pellets. Keys; ○: Simulation, ●: PEG 6000, ▲: DBP, □: TEC.

the diluent, Eudragit RS 100 containing PEG 6000 as the coating solution. The dissolution characteristics of the final formulation and the marketed formulation were compared while changing the buffer and the results are presented in Fig. 4. As can be observed, similar release patterns were noted between the two formulations that satisfactorily retarded the release to give the desired drug release profiles. From the *in vitro* dissolution data, kinetics of drug release was found to be zero-order. Dissolution rate constants showed no significant difference between the two formulations. Hence, it can be inferred that the developed formulation has "equivalent dissolution rate" to the marketed product of AT.

Bioavailability studies

In vivo evaluations of the developed formulation were carried out by estimating the plasma AT levels for a period up to 96 h using the marketed formulation as a reference. Studies in human volunteers revealed that the prepared

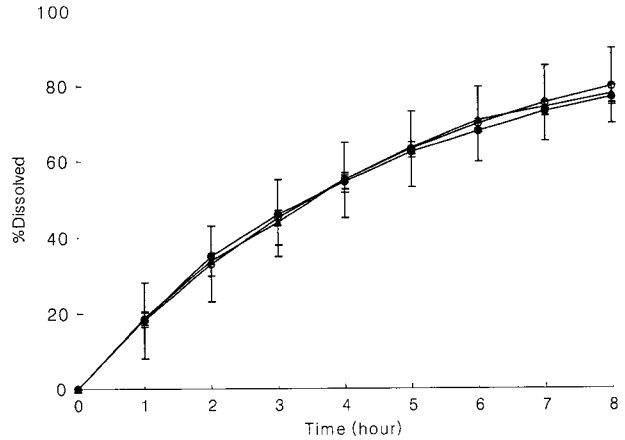

Fig. 4. Dissolution profiles of ATP and Saroten® retard in pH gradient conditions at 37°C. Keys; ○: Simulation, ●: ATP, ▲: Saroten® retard.

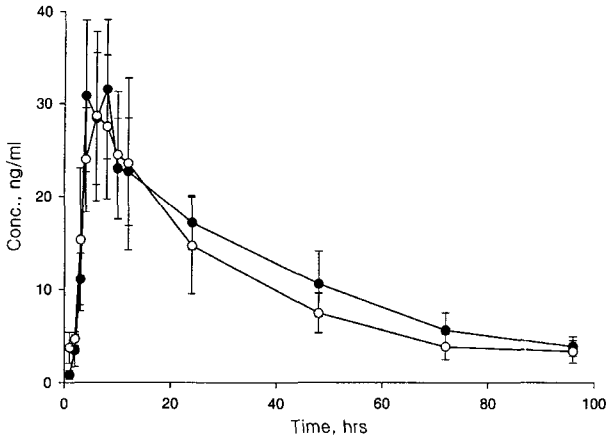

Fig. 5. Plasma concentrations of amitriptyline after oral administration of ATP (●) and Saroten® retard (○) to human volunteers under fasting conditions.

formulations exhibited an extended drug release pattern for a period of 96 h. Fig. 5 presents the plasma profiles of AT over 96 h after a single dose of 50 mg of AT (Saroten® retard or ATP) in 4 volunteers. The C_{max} were 36.4 and 29.7 ng/mL, and the mean $AUC_{0\text{-}96}$ were 1180.2 and 1010.7 ng · h/mL for ATP and Saroten® retard, respectively. The T_{max} was 6 h for both formulations.

Upon statistical evaluation, C_{max}, T_{max} and $AUC_{0\text{-}96}$ data did not show a significant difference within and between products ($p<0.05$). Thus, the newly developed ATP in this study may be bioequivalent to the product in the market.

CONCLUSIONS

The present study demonstrates that a controlled-release AT-loaded pellet formulation was successfully prepared using lactose as a diluent and Eudragit RS 100 incorporated with a plasticizer PEG 6000 as a coating agent. ATP prepared by the powder layering technique revealed better spherical shapes than those prepared by the solvent spraying technique as observed by scanning electron microscopy.

Bioavailability studies performed in human volunteers showed that *in vivo* plasma concentration profiles were comparable between ATP and Saroten® retard. Therefore, the newly developed ATP can be used as a possible alternative to the marketed product.

REFERENCES

Bechgaard, H., Brodie, R. R., Chasseaud, L. F., Houmoller, P., Hunter, J. O., Siklos, P., and Taylor, T., Bioavailability of indomethacin from two multiple-units controlled-release formulations. *Eur. J. Clin. Pharmacol.*, 21, 511-515 (1982).

Cole, G., Hogan, J., and Aulton, M., *Pharmaceutical Coating Technology*, Taylor and Francis, London, (1995).

Debbas, N., Ankier, S., and Warrington, S., Trazodone conventional and controlled-release formulations: pharmacodynamic effects after single and repeated administration. *Curr. Med. Res. Opin.*, 11, 501 (1989).

Ghahramani, P. and Lennard, M. S., Quantitative analysis of amitriptyline and nortriptyline in human plasma ans liver microsomal preparations by high-performance liquid chromatography. *J. Chromatogr. B.*, 685, 307-313 (1996).

Ghebre-Sellassi, I., *Multiarticulate Oral Drug Delivery*, Marcel Dekker, New York, (1994).

McGinity, J. W., *Aqueous Polymeric Coatings for Pharmaceutical Dosage Forms*, Marcel Dekker, New York, (1989).

Milada, D., On-line solid-phase extraction and high-performance liquid chromatographic determination of nortriptyline and amitriptyline in serum. *J. Chromatogr.*, 579, 291-297 (1992).

Peh, K. K., Yuen, K. H., Wong J. W., and Toh, W. T., Comparative bioavailability study of two atenolol tablet preparations. *Drug Dev. Ind. Pharm.*, 25, 357-360 (1999).

Pickup, A. J., Bojanowski, L. M., Dawling, S., Dinsdale, J., and Gosling, R. G., Comparison of the effect of amitriptyline in standard and sustained-release formulations on cardiac systolic time intervals. *J. Cardiovasc. Pharmacol.*, 4, 575-583 (1982).

Rolf, T. and Harald, O. B., Determination of amitriptylin oxide and its major metabolites amitriptyline and nortriptyline in plasma by high-performance liquid chromatography. *J. Chromatogr.*, 382, 372-376 (1986).

Yuen, K. H., Peh K. K., and Billa, N., Comparative bioavailability study of a generic naltrexone tablet preparation. *Drug Dev. Ind. Pharm.*, 25, 353-356 (1999).