Stability Study of Simvastatin under Hydrolytic Conditions Assessed by Liquid Chromatography

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In this work, a liquid chromatography stability-indicating method was developed and applied to study the hydrolytic behavior of simvastatin in different pH values and temperatures. The selected chromatographic conditions were a C18 column; acetonitrile–28 mM phosphate buffer solution, pH 4 (65 + 35) as the mobile phase; 251°C column temperature; and flow rate 1 mL/min. The developed method exhibited an adequate repeatability and reproducibility (coefficient of variation 0.54 and 0.74%, respectively) and a recovery higher than 98%. Furthermore, the detection and quantification limits were 9.1×10^{-7} and 2.8×10^{-6} M, respectively. The degradation of simvastatin fitted to pseudo-first order kinetics. The degradation was pH dependent, being much higher at alkaline pH than at acid pH. Activation energy, kinetic rate constants (k) at different temperatures, the half life ($t_{1/2}$) and the time for 10% degradation to occur (t_{90}) values are also reported.

Simvastatin—(+)–(1S,3R,7S,8S,8aR)-1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2-[(2R, 4R)-tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl]-1-naphthyl-2,2-dimethyl butanoate; Figure 1—is a well-known cholesterol-lowering agent belonging to the statin class, the most frequently prescribed and efficient drugs used to treat hypercholesterolemia and significantly reduce the morbidity and mortality associated with coronary heart disease (1, 2). Following oral administration, simvastatin is rapidly hydrolyzed in vivo to its corresponding β-hydroxy acid. The latter is a potent inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, an essential enzyme involved in the in vivo synthesis of cholesterol (3). The reductase inhibition is directly related to the structural similarity between the drug and the endogenous substrate; therefore, both the pharmacological and therapeutic activities of this drug have a close relationship with its structure. The basis of studies about the drug's stability from a structural point of view is the presence of some reactive centers, i.e., ester or lactone moieties, that can be hydrolyzed.

Investigation of the stability of drugs represents an important subject in drug quality evaluation. Many environmental conditions, such as heat, light, or the chemical susceptibility of substances to hydrolysis or oxidation, can have a very serious role in pharmaceutical stability (4, 5). Testing of a drug substance can help to identify likely degradation products and give important information on the drug's stability. Also, it can be an essential contribution to development and validation of stability indicating analytical methods used in monitoring the quality of pharmaceutical products. Independent of the final dosage form, forced degradation by exposure of a drug to different experimental conditions is useful to predict the potential degradation products. Hydrolysis is one of the most common degradation chemical reactions. Because water, either as a solvent or in the form of moisture in the air, contacts most pharmaceutical dosage forms to some degree, the potential for this degradation pathway exists for most drugs and excipients (6).

Simvastatin has been determined by several chromatographic methods, i.e., liquid chromatography (LC) with ultraviolet (UV) or fluorescence detection (7–11); LC/tandem mass spectrometry (LC/MS/MS; 12, 13); gas chromatography/MS (GC/MS; 11, 14–18), and LC coupled with atmospheric pressure ionization tandem MS (LC/API-MS/MS) in biological samples (12, 17, 19–23). On the other hand, micellar electrokinetic chromatography (24), LC-UV (25–27), and UV spectrophotometry have been used to determine simvastatin in both pure and dosage forms (26–29) and LC/electrospray ionization tandem MS (LC/ESI-MS/MS) has been used for determination in aqueous samples (30).

To date, no LC stability indicating analytical method has been described in the literature, and no previous systematic studies focused on simvastatin degradation have been performed. Only a short note about the influence of acid and alkali on simvastatin was published in addition to the LC/MS/MS analysis of this drug (12, 23). For this reason, this paper aimed to study the hydrolytic behavior of simvastatin at different pHs and temperatures and to develop an LC method for determination of simvastatin in the presence of its hydrolytic degradation product(s). The novelty of this work is based on description of a new analytical method that is

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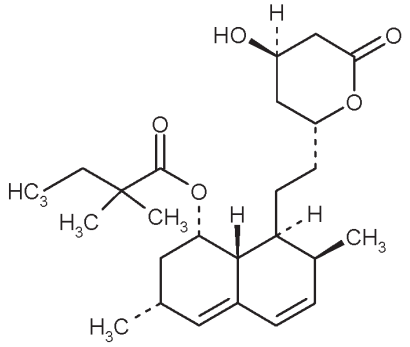

Figure 1. Chemical structure of simvastatin.

suitable for monitoring the purity of drug substance. The obtained results can be helpful to ensure the quality, safety, and effectiveness of pharmacotherapy.

Experimental

Reagents and Drugs

Simvastatin (99.9% chromatographically pure) was obtained from Ruibang Laboratories (Wenzhou, Zhejiang, China). All other reagents used were of analytical or LC grade. The water was double-distilled and deionized (Milli-Q quality).

Simvastatin Stock Solution

Simvastatin pure drug was weighed and dissolved in acetonitrile to obtain a concentration of 1.0×10^{-2} M. This solution was stable for at least 1 week when stored at 8°C.

Buffer Solutions

Phosphate buffer solution (28 mM) adjusted to pH 4 with 30% phosphoric acid or 30% NaOH solution was used as the mobile phase for LC experiments. For the degradation trials, 28 mM phosphate buffer solutions adjusted to different pH values (2–9) with 30% phosphoric acid or 30% NaOH solutions were used.

LC

LC measurements were carried out by using a Waters (Milford, MA) assembly equipped with a Model 600 controller pump and a Model 996 photodiode array detector. The data acquisition and treatment were made by means of Millenium version 2.1 software. A μBondapak/Porasil C18 analytical column, 10 μm particle size (3.9 × 150 mm) and a μBondapak C18 guard column (30 × 4.6 mm) were used. The injector was a 20 μL Rheodyne valve.

Chromatographic Conditions

The isocratic mobile phase was composed of acetonitrile–28 mM phosphate buffer, pH 4 (65 + 35) at 25°C with a flow of 1 mL/min. UV detection was at 238 nm.

LC System Suitability Test

A 1.0×10^{-4} M simvastatin solution (acetonitrile–28 mM buffer phosphate solution, pH 7) was heated at 80°C for 1 h to obtain the peaks corresponding to both simvastatin and its degradation product. This sample was used to perform the system suitability test. The effects of different concentrations of acetonitrile (40, 50, 60, 70, and 80%) on the capacity factor (k′), resolution (R), relative retention (α), and tailing factor (T) were tested to determine the best analytical conditions (27, 31).

Degradation Trials

Phosphate buffer solutions (28 mM) at pH 2, 3, 4, 5, 6, 7, 8, and 9 were spiked with simvastatin to obtain an initial concentration ranging between 1.0×10^{-3} and 5.0×10^{-5} M in a 50% acetonitrile–50% phosphate buffer solution. Solutions were divided into a number of 2 mL amber vials (at least 2 for each point of the degradation curve) and then placed in an oven at 80, 60, and 40°C (±0.2°C). Vials were taken from the oven at selected time intervals depending on pH (20 and 60 min for pH 7–8 and 2–6, respectively). Immediately, each sample was cooled on ice to quench the reaction and assayed by LC. Experiments were carried out in duplicate, and the degradation was monitored over at least 3 half-lives.

Thin-Layer Chromatography (TLC)

TLC experiments were carried out using silica gel F254 plates as the stationary phase and ethyl acetate as the mobile phase. Bromocresol green (50 mg in 250 mL ethanol +2 mL 0.1 M NaOH) was used as detection reagent (32). Aliquots (5 μL) of 1×10^{-4} M simvastatin standard solution in acetonitrile and samples from the hydrolysis trials were chromatographed.

Activation Energy (Ea)

Each Ea value was obtained from the Arrehnius model by plotting ln k vs 1/T for each concentration tested. The final Ea value represents the average of the Ea calculated for 4 concentrations between 1×10^{-3} and 1×10^{-5} M. In all cases, regression coefficient values higher than 0.997 were obtained.

Results and Discussion

The effect of the concentration of acetonitrile on the k′ value of simvastatin and its hydrolytic degradation product is shown in Figure 2A. As can be seen, the k′ values decreased as the concentration of acetonitrile increased, with the k′ of simvastatin decreasing at higher rate than that of its degradation product. Resolution changed with a pattern similar to k′ but significantly decreased from 70% acetonitrile. Furthermore, α remained relatively constant but decreased from 70% acetonitrile. On the other hand, the tailing factor of both substances decreased as the percentage of acetonitrile increased, and the retention times were shortened as a function of increasing percentage of acetonitrile. These effects are shown in Figure 2(B). Taking into account these results, the

selected optimal conditions were acetonitrile–28 mM phosphate buffer, pH 4 (65 + 35); 25°C; and 1 mL/min flow rate.

In Figure 3, typical chromatograms of standard simvastatin and samples after hydrolysis at different pH values with the final selected conditions are shown. Simvastatin exhibited a retention time (Rt) of 8.27 ± 0.04 min [Figure 3(A)], and its corresponding degradation product had an Rt of 4.50 ± 0.23 min, either from acid [Figure 3(B)] or alkaline hydrolysis [Figure 3(C)]. Simvastatin degradation product retains the original UV spectrum of the parent drug, indicating that the chromophore structure remains unaltered and that only the ester or lactone moiety is affected by the hydrolysis processes. This observation is in line with previous reports in which only one degradation product was obtained after the simvastatin hydrolysis. These authors concluded that the

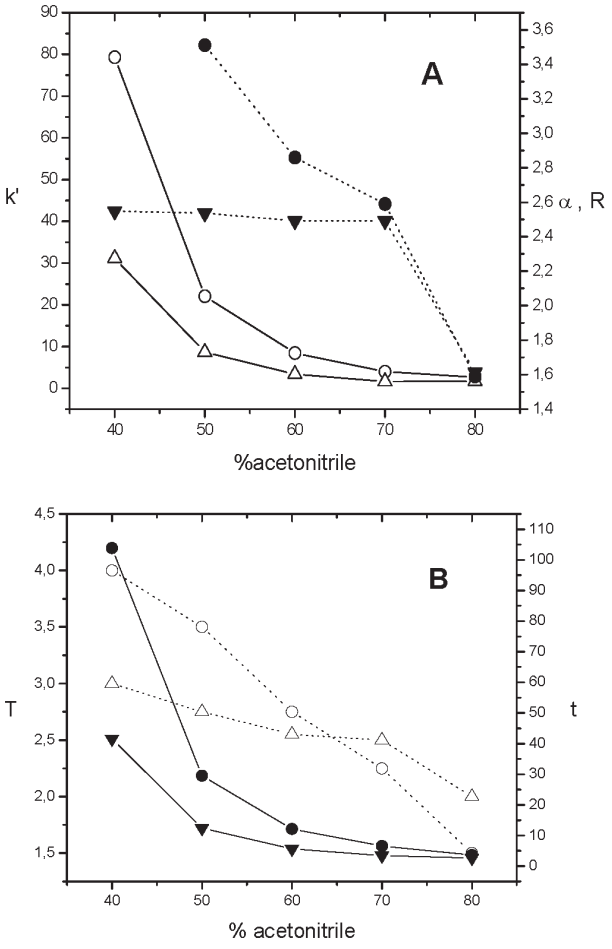

Figure 2. Dependence of the percentage of acetonitrile on (A) k′ values of simvastatin (○) and its degradation product (△), R values (●), and α values (▼); (B) tailing factor (T) of simvastatin (○) and its degradation product (△) and retention time (t) of simvastatin (●) and its degradation product (▼). All mobile phases were acetonitrile–28 mM phosphate buffer, pH 4.

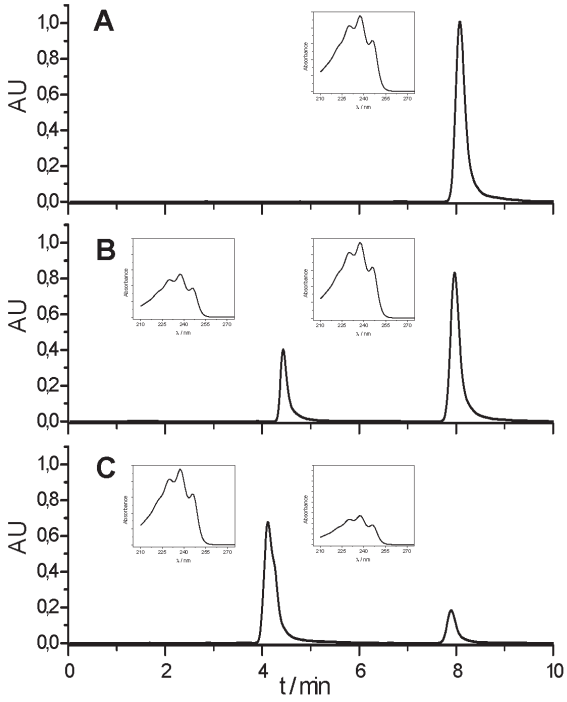

Figure 3. Chromatograms of a 5×10^{-4} M simvastatin standard solution at *t* = 0 min (A), after 9 days of hydrolysis at pH 3 and 60°C (B) and 1.5 h of hydrolysis at pH 8 and 60°C (C). UV spectrum of each peak.

degradation product corresponds to the opening of the lactone ring (12, 20). From the TLC experiments, it was found that standard simvastatin and its degradation product generated from the hydrolysis trials exhibited R^f values of 0.82 and 0.57, respectively. By using bromecresol green as the detection reagent, only one yellow spot was observed at R_f 0.57. This was evidence of the presence of an acid moiety in the degradation product which is consistent with the previous assumption that in this product, the lactone moiety has been broken to give the carboxylic acid derivative.

In the present study, experiments were conducted at 40, 60, and 80°C. As expected, and parallel with the time-course of the hydrolysis, a decrease of the original peak corresponding to the parent drug occurred without interference from other signals.

In Table 1, the analytical assessment of the new LC procedure for simvastatin is summarized. From the obtained results, it can be concluded that the developed chromatographic assay fulfills the analytical requirements, exhibiting an adequate repeatability and reproducibility [coefficient of variation (CV) 0.54 and 0.74%, respectively] and a recovery higher than 98% (33). On the other hand, the concentration range for calibration graphs seems to be adequate to follow degradation, with detection and quantification average limits of 9.1×10^{-7} and 2.8×10^{-6} M, respectively. The limit of detection (LOD) and limit of quantification (LOQ) values of the method were calculated by using the average (*Yb*) and standard deviation (*Sb*) of the blank estimated response and calibration graph slopes (*m*),

Table 1. Analytical parameters of the developed LC–UV method with detection at 238 nm

Parameter	
Repeatability, CV (%)[a]	0.54
Reproducibility, CV (%)[a]	0.74
Recovery (%)[b] ± standard deviation	98.6 ± 0.9
Concentration range (M)	1.0×10^{-6} to 1.0×10^{-3}
Calibration plot	AUC = 3.17 × 1010[c] + 2361.24 (r = 0.99998, n = 7)
Detection limit (M)	9.1×10^{-7}
Quantification limit (M)	2.8×10^{-6}

[a] Concentration level of 5×10^{-5} M.

[b] Average for a concentration level of 5×10^{-5} M.

with signal/noise ratios of 3 and 10, respectively, according to the following expressions (31):

$$\text{LOD} = \left[\frac{(Yb + 3 \cdot SB)}{m}\right] \text{ and } \text{LOQ} = \left[\frac{(Yb + 10 \cdot Sb)}{m}\right]$$

Thus, the developed method was found to be sufficiently selective to discriminate simvastatin from the corresponding hydrolysis product; it represents a useful tool to follow this type of degradation and to assess simvastatin in the presence of its active metabolite.

The developed method was successfully applied to determine the stability of simvastatin at different pHs and temperatures. First of all, experiments under constant oxygen bubbling for 3 h at different pHs (4, 7, and 10) were carried out. Results from these studies indicated that simvastatin was not oxidized under these experimental conditions. Consequently, a nitrogen atmosphere was not necessary to perform the degradation trials. To test the kinetic order of the hydrolytic degradation, experiments with both different initial concentration (C) and pH were performed. As can be seen from Figure 4, the ln C versus time (t) plots were linear (r > 0.9991), and the changes in the initial concentration of simvastatin did not affect the slopes of the decay curves. Furthermore, all of the plots were parallel, and their slopes were found not to be statistically different after applying the F-test (variance proportion) and the t-test (P = 0.05, n = 8). Therefore, a pseudo–first order kinetics for the hydrolytic degradation of simvastatin can be assumed (34).

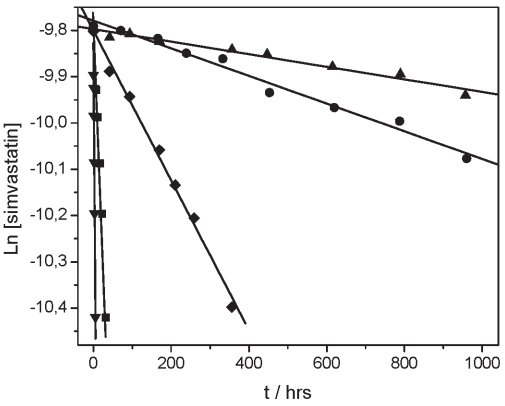

Figure 5. Effect of pH on 5.5×10^{-5} M simvastatin concentration at 40°C (▲ pH 3, ● pH 5, ▲ pH 6, ! pH 7, ▼ pH 8).

Degradation of simvastatin was dramatically influenced by both pH and temperature. As can be seen in Figure 5, at 40°C, k values varied 5.4- and 66-fold between pH 5–6 and 6–8, respectively. Due to the rapid decay of simvastatin concentration at pH 8, the experimental data points were determined only within the first 10 h of the degradation. The instability of simvastatin increased concomitantly with increasing pH, and from pH 9 the hydrolysis became spontaneous even at room temperature. This behavior is summarized in Figure 6, in which the pH hydrolysis rate profile for the simvastatin at 60°C is presented. As can be seen, k values below pH 6 are relatively small and constant,

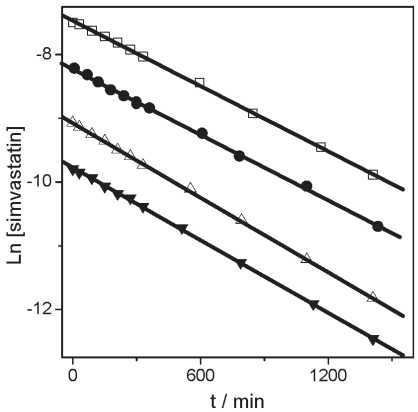

Figure 4. Ln C vs t-plots of simvastatin hydrolysis at pH 7 and 60°C (□ 5×10^{-4} M, ● 2.5×10^{-4} M, △ 1.0×10^{-4} M, ▼ 5×10^{-5} M).

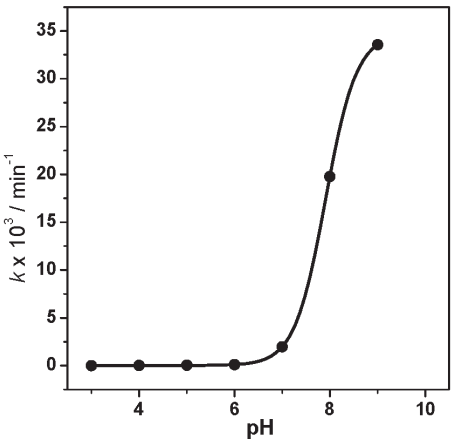

Figure 6. pH-rate of hydrolysis profile of simvastatin at 60°C.

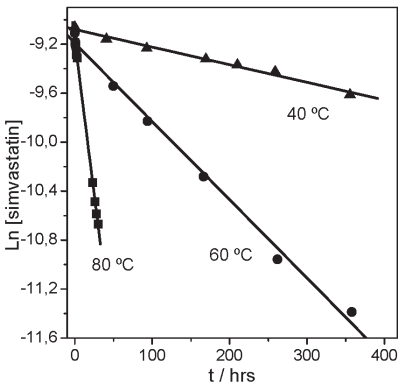

Figure 7. Effect of temperature on 1×10^{-4} M simvastatin concentration at pH 6.

and then they exhibit an exponential increase in the range between pH 6 to 9.

Similar effects were obtained when simvastatin was exposed at different temperatures; *k* values increased 4.4- and 7.9-fold parallel with the increase of temperature from 60 to 80°C at pH 6 (Figure 7). The calculated activation energy at pH 6 (60°C) was 19.9 Kcal/mol, a value that is consistent with the dissociation of ester or lactone moieties (34). Furthermore, the enthalpy had a value of 19.2 Kcal/mol and the free energy had a value of –25.5 Kcal/mol. The value of $\triangle G < 0$ implied that the hydrolysis under these experimental conditions was spontaneous.

In Table 2, both the kinetic parameters at 60°C and the extrapolated values at 25°C are summarized. From the extrapolated data at 25°C, it can be seen that at pH 8 a 10% decrease in the initial concentration occurred within 8.6 h. In contrast, at pH 7 simvastatin was 22-fold more stable than at pH 8. Furthermore, simvastatin increased its stability at acidic pHs, i.e., at pH 5 the stability increased 160-fold compared with pH 8.

Conclusions

The following are the conclusions of this study: after acid or basic hydrolysis of simvastatin, one product was generated with an Rt of 4.50 ± 0.23 min; the UV spectra of the degradation products was similar to the original spectrum, revealing that the chromophore structure of simvastatin was not affected by the hydrolysis; the LC method fulfills analytical requirements of an adequate repeatability and reproducibility; the degradation of simvastatin fits pseudo-first order kinetics and increases with temperature; the calculated activation energy at pH 6 (60°C) was 19.9 Kcal/mol, supporting the dissociation of the lactone moiety; and the hydrolysis of simvastatin is pH dependent, being greater at alkaline pH compared to acid pH.

Table 2. Kinetic parameters at 60°C and extrapolated to 25°C

	T = 60°C		*T* = 25°C		
pH	k (min^{-1})	$t_{1/2}$ (h)	k (min^{-1})	$t_{1/2}$ (h)	t_{90} (h)
5	2.16×10^{-5}	534.8	1.28×10^{-6}	9058.8	1372.6
6	1.12×10^{-4}	103.4	3.90×10^{-6}	2965.3	449.3
7	1.78×10^{-3}	6.5	9.22×10^{-6}	1252.7	189.8
8	1.96×10^{-2}	0.59	2.03×10^{-4}	56.95	8.63

Acknowledgments

The authors are very grateful for the support of FONDECYT Grant No. 1030737.

References

(1) Hoffman, W.F., Alberts, A.W., Anderson, P.S., Smith, R.L., & Willard, A.K. (1996) *J. Med. Chem.* **29**, 849–852

(2) Kaufman, D.W., Kelly, J.P., Rosenberg, L., Anderson, T.E., & Mitchell, A.A. (2002) *J. Am. Med. Assoc.* **287**, 337–344

(3) Vickers, S., Duncan, C.A., Chen, I.W., Rosegay, A., & Duggan, D.E. (1990) *Drug Metab. Dispos*. **18**, 138–145

(4) *Stability Testing of New Drugs Substances and Products* (2002) Proceedings of the International Conference on Harmonization (ICH), IFPMA, Geneva, Switzerland

(5) Kommanaboyina, B., & Rhodes, C.T. (1999) *Drug. Dev. Ind. Pharm*. **25**, 857–868

(6) Waterman, K.C., Adami, C.R., Alsante, K.M., Antipas, A.S., Arenson, D.R., Carrier, R., Hong, J., Landis, M.S., Lombardo, F., Shah, J.C., Shalaev, E., Smith, S.W., & Wang, H. (2002) *Pharm. Dev. Technol*. **7**, 113–146

(7) Bologna, M., Carlucci, G., Biordi, L., Mazzeo, P., & Iannarelli, R. (1994) *Int. J. Immunopathol. Pharmacol*. **7**, 79–85

(8) Carlucci, G., Mazzeo, P., Biordi, L., & Bologna, M. (1992) *J. Pharm. Biomed. Anal*. **10**, 693–697

(9) Ochiai, H., Uchiyama, N., Imagaki, K., Hata, S., & Kamei, T. (1997) *J. Chromatogr. B* **694**, 211–217

(10) Kim, B.C., Ban, E., Park, J., Song, Y.K., & Kim, C.K. (2004) *J. Liq. Chromatogr. Relat. Technol.* **27**, 3089–3102

(11) Erturk, S., Onal, A., & Cetin, S.M. (2003) *J. Chromatogr. B* **793**, 193–205

(12) Zhao, J.J., Xie, I.H., Yang, A.Y., Roadcap, B.A., & Rogers, J.D. (2000) *J. Mass Spectrom*. **35**, 1133–1143

(13) Walsky, R.L., Gaman, E.A., & Obach, R.S. (2005) *J. Clin. Pharmacol.* **45**, 68–78

(14) Pfohl, M., Naoumova, R.P., Kim, K.D., & Thompson, G.R. (1998) *Eur. J. Clin. Invest.* **28**, 491–496

(15) Lilja, J.J., Kivisto, K.T., & Neuvonen, P.J. (1995) *Clin. Pharmacol. Ther.* **64**, 477–483

(16) Watts, G.F., Cummings, M.H., Umpleby, M., Quiney, J.R., Naoumova, R., Thompson, G.R., & Sonksen, P.H. (1995) *Eur. J. Clin. Invest*. **25**, 559–567

(17) Morris, M.J., Gilbert, J.D., Hsieh, J.Y., Matuszewski, B.K., Ramjit, H.G., & Bayne, W.F. (1993) *Biol. Mass Spectrom*. **22**, 1–8

(18) Yang, H.T., Feng, Y., & Luan, Y.W. (2003) *J. Chromatogr. B* **785**, 369–375

(19) Jemal, M., Ouyang, Z., & Powell, M.L. (2000) *J. Pharm. Biomed. Anal*. **23**, 323–340

(20) Jemal, M., & Ouyang, Z. (2000) *Rapid Commun. Mass Spectrom*. **14**, 1757–1765

(21) Zhao, J.J., Yang, A., & Rogers, J.D. (2002) *J. Mass Spectrom*. **37**, 421–433

(22) Wu, Y., Zhao, J.J., Henion, J., Korfmacher, W.A., & Lin, C.C. (1997) *J. Mass Spectrom*. **32**, 379–387

(23) Zhang, N., Yang, A., Rogers, J.D., & Zhao, J.J. (2004) *J. Pharm. Biomed. Anal.* **34**, 175–187

(24) Srinivasu, M.K., Raju, A.N., & Reddy, G.O. (2002) *J. Pharm. Biomed. Anal.* **29**, 715–721

(25) Godoy, R., Godoy, C.G., De Diego, M., & Gomez, C. (2004) *J. Chil. Chem. Soc.* **49**, p. 289

(26) Carlucci, G., & Mazzeo, P. (1992) *Farmaco* **47**, 817–823

(27) *U.S. Pharmacopeia and 27 National Formulary* (2004) U.S. Pharmacopeial Convention, Rockville, MD, pp 1692, 2281

(28) Erk, N. (2002) *Pharmazie* **57**, 817–819

(29) Wang, L., & Asgharnejad, M. (2000) *J. Pharm. Biomed. Anal.* **21**, 1243–1248

(30) Miao, X., & Metcalfe, C.D. (2003) *J. Chromatogr. A* **998**, 133–141

(31) Quattrochi, O.A., De Andrizzi, S.A., & Laba, R.F. (1992) *Introducción a la HPLC, aplicación y práctica*, Artes Gráficas Farro SA, Buenos Aires, Argentina, p. 279

(32) *Clarke's Analysis of Drugs and Poisons* (2003) A.C. Moffat, M.D. Osselton, & B. Widdop (Eds), Pharmaceutical Press, London, UK, p. 392

(33) *AOAC Peer Verified Methods Program, Manual on Policies and Procedures* (1993) AOAC INTERNATIONAL, Gaithersburg, MD

(34) Connors, K.A., Amidon, C.L., & Stella, V.J., (1986) *Chemical Stability of Pharmaceuticals: A Handbook for Pharmacist*s, Wiley-Interscience, New York, NY