ISSN 1061-9348, Journal of Analytical Chemistry, 2014, Vol. 69, No. 4, pp. 384–389. © Pleiades Publishing, Ltd., 2014.

ARTICLES

# Stability Indicating RP-HPLC Method for Simultaneous Estimation of Rupatadine Fumarate and Montelukast Sodium in Bulk and Tablet Dosage Form[1]

**Vivekkumar K. Redasani, Amit R. Kothawade, and Sanjay J. Surana**

*Department of Pharmaceutical Chemistry, R. C. Patel Institute of Pharmaceutical Education and Research Shirpur, Dist: Dhule (MS) 425 405 India*

Received July 22, 2011; in final form, July 4, 2012

**Abstract**—A simple, selective, accurate and sensitive high performance liquid chromatography (**HPLC**) method was developed and validated for the simultaneous determination of rupatadine fumarate (**RPT**) and montelukast sodium (**MNT**). Chromatographic separation achieved isocratically on a Hypersil BDS $C_8$ (250 mm × 4.6 mm, 5 μm) column utilizing a mobile phase of methanol: acetonitrile: buffer (40 : 30 : 30), (pH 3 with $H_3PO_4$) at a flow rate of 1.0 mL/min and column oven temperature 40°C with UV detection at 270 nm. Statistical analysis proves that the method is reproducible and selective for the simultaneous estimation of RPT and MNT. As the method could effectively separate the drugs from their degradation products, it can be employed as stability indicating method. The developed method was validated as per ICH guidelines in terms of accuracy, precision, linearity and specificity.

*Keywords:* rupatadine fumarate, montelukast sodium, RP-HPLC, validation, degradation study

**DOI:** 10.1134/S1061934814040121

Rupatadine fumarate (Fig. 1a) is 8-chloro-6,11-dihydro-11-(1-((5-methyl-3-pyridyl)methyl)-4-piperidylidene)-5*H*-benzo(5,6)cyclohepta(1,2-b)pyridine. RPT is a non-sedating H1 antihistamine that further blocks platelet-activating factor (**PAF**) receptor. It is a potent and orally active therapeutic agent for the treatment of seasonal allergic rhinitis and chronic idiopathic urticaria [1].

Montelukast sodium (Fig. 1b) is 2-[1-[1(R)-[3-[2(E)-(7-chloroquinolin-2-yl)vinyl]phenyl]-3[2-(1-hydroxy-1-methylethyl)phenyl]propylsulfanylmethyl]cyclopropyl] acetic acid sodium salt. MNT is a specific cysteinyl leukotriene receptor antagonist belonging to a styryl quinolines series. It is agent of choice in various cases for the treatment of bronchial asthma [2]. RPT and MNT is a well accepted combination in treatment of asthma, allergic rhinitis and urticaria. Presence of RPT also enhances the effect of MNT in asthma.

Various methods of analysis were documented for the determination of RPT and MNT individually. RPT was determined in pharmaceutical formulation by HPLC [3], LC–MS/MS [4] and HPTLC [5]. Methods available for the separation and individual determination of MNT include HPLC [6], protein precipitation [7], LC–MS/MS [8], liquid–liquid extraction using HPLC with fluorescence detection [9], stereo-selective HPLC using column-switching [10], and determination in human plasma by the column-switching HPLC [11], derivative spectroscopy HPLC [12], microwave-assisted extraction [13], pressurized liquid extraction form pharmaceutical solid dosage form [14], residual determination in bulk drug [15].

Apart from individual analyses, a spectrophotometric method for simultaneous estimation of RPT and MNT was also reported [16]. This indicates that for such a widely used combination of drugs only methods of determination of individual compounds are reported and no HPLC method has been developed for the simultaneous determination of this combination in commercial formulations. This fact stimulated us to carry out this particular work. Moreover, validation and stability indicating studies in combination also proves whether both drugs have chemical interaction between them or not. Thus, it would be beneficial to provide accurate, precise and reliable method for simultaneous determination of RPT and MNT. The present work describes analytical procedures for the quantitation of RPT in co-formulation with MNT using reversed phase HPLC.

## EXPERIMENTAL

**Solvents and reagents.** RPT and MNT were obtained as a gift from Zydus Cadila Ltd., Ahmedabad

[1] The article is published in the original.

(India). Rupanex M tablets (Dr. Reddy's Lab.) were procured from local market. HPLC grade acetonitrile and methanol were procured from Merck, India. Potassium dihydrogen orthophosphate and orthophosphoric acid AR grade were from Merck, India. Highly pure water was prepared by using Millipore Milli Q plus purification system.

**Instrumentation and materials.** Analysis was performed on Waters HPLC 2695 separation module with built-in PDA detector and auto sampler. Chromatographic software Empower 2 was used for data collection and processing. The analytical column was Hypersil BDS $C_8$ (250 mm × 4.6 mm, 5 μm) with the mobile phase methanol: acetonitrile: phosphate buffer (40 : 30 : 30, v/v), pH of buffer was maintained at $3 \pm 0.05$ with $H_3PO_4$. Mobile phase was filtered with 0.22 μm filter in Millipore vacuum filtration assembly and degassed prior to operating under isocratic condition at a flow rate of 1.0 mL/min. Sample injection volume was 20 μL and column oven temperature was 40°C, elution was monitored at 270 nm with run time 20 min.

**Sample preparation.** Stock solutions (10 μg/mL) of RPT and MNT were prepared in HPLC-grade methanol : water (80 : 20, v/v). The solutions were kept in dark until analysis. Series of each standard were prepared by progressive dilution of the stock solution.

**Linearity.** Appropriate aliquots of standard stock solution were taken in different 10mL volumetric flasks and diluted up to the mark with mobile phase to obtain final concentrations of 5, 8, 10, 12 and 15 μg/mL of each compound. The solutions were injected using a 20 μL fixed loop system and chromatograms were recorded. RPT and MNT both follow linearity in range 5 to 15 μg/mL (Table 1).

**Analysis of the tablet dosage form.** Twenty tablets (Rupanex-M) were weighed accurately and crushed to form fine powder. Powder weight equivalent to 20 mg of RPT and MNT each were dissolved in a 200 mL volumetric flask with methanol. It was sonicated followed by filtration using Whatmann filter paper No. 1. Appropriate aliquots were transferred into five different 50 mL volumetric flasks and the volume was made up to the mark with methanol: water (80 : 20, v/v) to get concentration 10 μg/mL. The solutions were subject to analysis and results obtained as in Table 2.

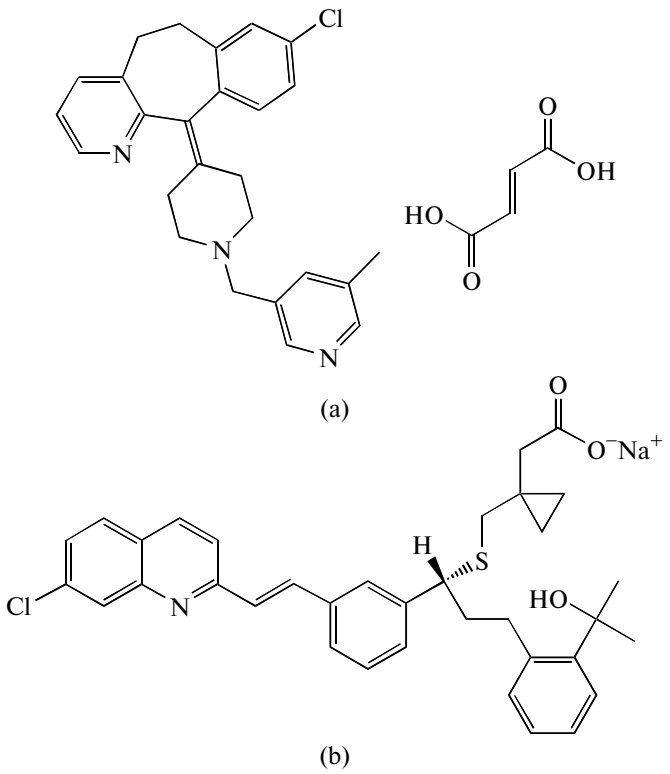


**Fig. 1.** Chemical structures of RPT (a) and MNT (b).

**Validation parameters.** The developed method was validated as per ICH guidelines in terms of its linearity, accuracy, limit of detection (**LOD**), limit of quantification (**LOQ**), specificity, intra-day and inter-day precision and repeatability of measurement.

**Accuracy.** It was found out by recovery study using standard addition method. Known amounts of standards of RPT and MNT were added to pre-analyzed samples at a level from 80 up to 120% and then subjected to the proposed HPLC method. Results of recovery studies are shown in Table 3.

**Precision.** Intraday and interday precision of the assay of samples containing RPT and MNT having concentration 8, 10, 12 μg/mL for both were analyzed five times in the same day (intraday) and for three con-

**Table 1.** Linearity of the proposed method for RPT and MNT ($n = 5$)

| Parameter | RPT | MNT |
|---|---|---|
| Linear range, mg/mL | 5–15 | 5–15 |
| Slope | $2.6 \times 10^4$ | $4.14 \times 10^4$ |
| Intercept | −3150 | −12314 |
| Correlation coefficient ($r^2$) | 0.9997 | 0.9995 |

**Table 2.** Analysis of tablet formulation ($n = 5$)

| Analyte | Label claim, mg/tablet | Amount found, mg/tablet | RSD, % |
|---|---|---|---|
| RPT | 10 | 10.0 | 0.4 |
| MNT | 10 | 10.1 | 0.6 |

**Table 3.** Recovery studies

| Level of recovery | Amount of drug added | | Recovery, % | | RSD, % | |
|---|---|---|---|---|---|---|
| | RPT | MNT | RPT | MNT | RPT | MNT |
| 80% | 8 | 8 | 100.4 | 99.4 | 0.7 | 1.2 |
| 100% | 10 | 10 | 100.1 | 99.9 | 0.9 | 0.3 |
| 120% | 12 | 12 | 99.1 | 99.9 | 0.5 | 0.7 |

secutive days by different analysts (interday). Precision was calculated as intra and interday coefficient of variation [% C.V. = (S.D./mean) × 100], as shown in the Table 4.

**Robustness.** By introducing deliberate small changes in the mobile phase pH (±0.2), flow rate (±0.1 mL/ min), temp. (±5°C), and robustness of the proposed method was studied.

**Limit of detection and limit of quantification.** The LOD and LOQ were calculated by the use of the equations LOD = 3.3 × $N/B$ and LOQ = 10 × $N/B$, where $N$ is the standard deviation of the peak areas of the drug ($n = 3$), taken as the measure of the noise, and $B$ is the slope of the corresponding calibration plot. The signal to noise ratio was determined. The LOD was regarded as the amount for which the signal to noise ratio was 3 : 1 and LOQ regarded as the amount for which the signal to noise ratio was 10 : 1 Results are shown in Table 4.

**Table 4.** Summary of validation data

| Parameter | RPT | MNT |
|---|---|---|
| Linear range, μg/mL ($n = 5$) | 5–15 | 5–15 |
| Correlation coefficient ($r^2$) | 0.9997 | 0.9995 |
| Limit of detection, μg/mL | 0.68 | 0.89 |
| Limit of quantification, μg/mL | 1.85 | 2.32 |
| % Recovery ($n = 9$) | 99.1 to 100.3 | 99.8 to 99.9 |
| Precision, RSD, % ($n = 5$) | | |
| Repeatability | 0.561 | 0.856 |
| Intra-day | 0.658 | 0.725 |
| Inter-day | 0.797 | 0.976 |
| Robustness | Robust | Robust |

**Forced degradation studies.** Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method. Intentional degradation was carried out by exposing 20 mg of samples in three 200 mL flasks containing acid (0.1 M HCl at 60°C), base (0.1 M NaOH at 60°C) and hydrogen peroxide (3% $H_2O_2$) for 30 min while one volumetric flask was exposed to light for 12 h. Acidic and basic solutions were neutralized. Then 5 mL aliquots were transferred into three other 50 mL volumetric flasks and made up to the mark with methanol: water (80 : 20, v/v) in order to get the concentration of 10 mg/mL. All these three volumetric flasks were kept in dark to exclude the possible degradation effect of light. Twenty μL of sample solutions were injected and analyzed against control samples (lacking of degradation treatment). MNT is found to be degraded in light, acid and hydrogen peroxide but no interferences of their degradants (by checking peak purity) and excipients was observed (by checking against placebo) (Table 5).

## RESULTS AND DISCUSSION

An RP-HPLC method was optimized with a view to develop an accurate and reproducible method so as to resolve drugs. Isocratic elution is simple, requires only one pump and flat baseline separation is achieved for easy and reproducible results.

Optimization of the method was done by altering mobile phase composition, pH, column packing, flow rate, temperature, detection wavelength, and the effects on retention and peak shape were monitored for RPT and MNT. The final chromatographic conditions have been set for stationary phase giving satisfactory resolution and run time with reversed phase Hypersil BDS $C_8$ (250 mm × 4.6 mm, 5 μm particle diameter) column. A series of mobile phases varying the pH and volume fractions of acetonitrile and methanol have been also tested and the best results obtained by use of

**Table 5.** Forced degradation study

| Agent | Exposure time | Condition | Degradant peak | | RT, min | | Degradation, % | |
|---|---|---|---|---|---|---|---|---|
| | | | RPT | MNT | RPT | MNT | RPT | MNT |
| 0.1 M HCl | 30 min | 60°C | – | 2 | – | 24.8 and 30.2 | 0.3 | 28.7 |
| 0.1 M NaOH | 30 min | 60°C | – | – | – | – | 2.9 | 2.4 |
| 3% $H_2O_2$ | 30 min | 60°C | – | 1 | – | 7.7 | 4.5 | 24.4 |
| Light | 12 h | Sunlight | – | 2 | – | 7.5 and 11 | – | 10.6 |

**Table 6.** System suitability ($n = 5$)

| Parameter | Acceptance criteria | Result | |
|---|---|---|---|
| | | RPT | MNT |
| Theoretical plates (number) | More than 2000 | $6.3 \times 10^3$ | $1.2 \times 10^4$ |
| USP tailing factor | Less than 2 | 1.4 | 1.2 |
| Capacity factor | Should be non-zero | 0.64 | 4.31 |
| USP resolution | More than 2 | 31.7 | |

mobile phase consisting of methanol: acetonitrile: buffer (pH 3) in 40 : 30 : 30 giving well resolved, sharp peaks for RPT and MNT with a retention time of 3.9 and 15.4, respectively (Fig. 2). The flow rate of 1.0 mL/min at 270 nm and 40°C temperature for column oven was found to be the best for analysis. System suitability parameters were studied by injecting five replicate injections of working standard solution (10 μg/mL) (Table 6). RSD was less than 2% in intraday and interday precision and in each parameter of robustness. So the proposed method is precise and robust.

The forced degradation studies of RPT and MNT was done to observe the number of degradants and their retention times. This would also help to check the interference of degradants and excipients in the combination of drugs. The results of specificity studies indicated no interference from excipients, impurities and degraded products due to various stress conditions. This assures that the peak response was due to a single component. RPT does not show any peaks of degradants as it degraded negligibly in all conditions. The percentage degradation was found to be less than 10%, which may not be accountable as per ICH guidelines. MNT contains a thiol group and hence its degradation in light and peroxide can be predicted. Apart from this, MNT degrades in acidic condition when kept in 0.1 M HCl for 30 min, and two degradant peaks separate out at 24.8 and 30.2 min, respectively (Fig. 3a), while no degradation is seen in basic condition. Oxidative degradation is seen when kept in $H_2O_2$ for 30 min, which gives single degradant peak at 7.7 min (Fig. 3b), and

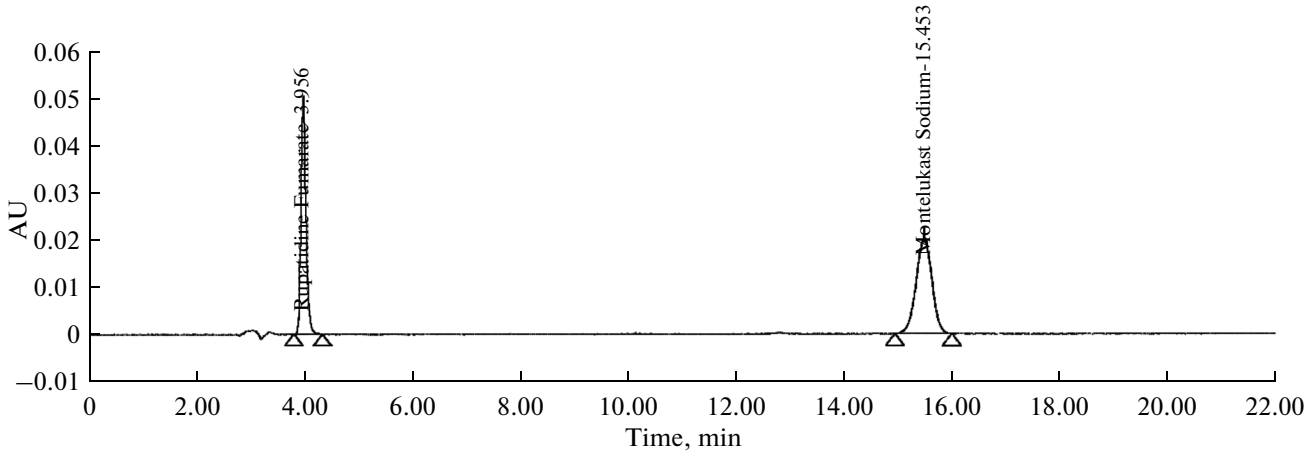


**Fig. 2.** Typical chromatogram of RPT and MNT with retention time 3.9 and 15.4 min, respectively.

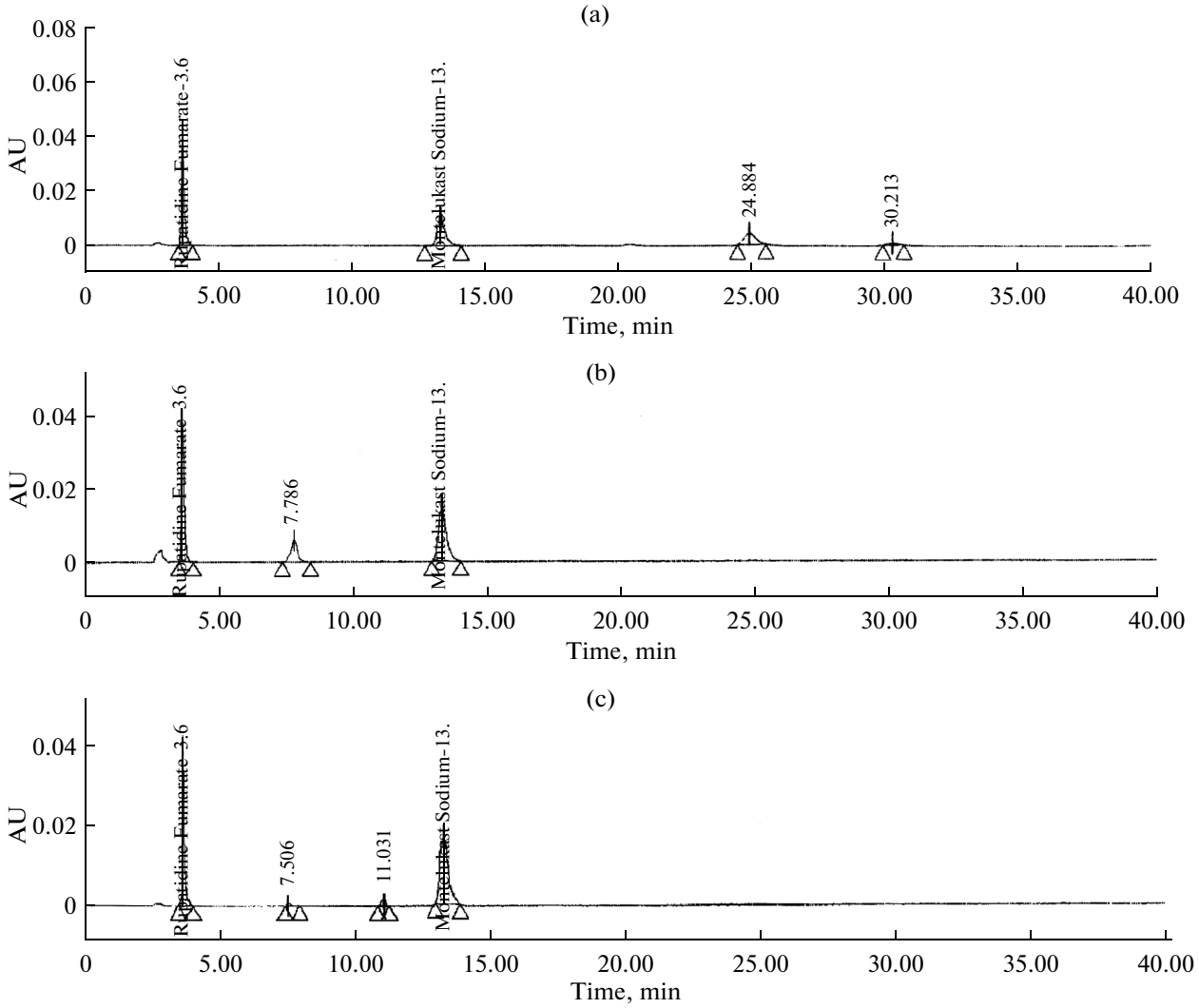


**Fig. 3.** Degradation of RPT and MNT in acid (a), peroxide (b), and light (c).

two peaks are obtained at 7.5 and 11 min when exposed to light Fig. 3c).

* * *

The proposed method was validated as per ICH guidelines by preliminary analysis of standard sample and recovery studies. The percentage of average recoveries for RPT and MNT obtained was 99.84 and 99.71, respectively. From the degradation study it was concluded that MNT is more sensitive to light and peroxide and it gives two degradants in acid, while RPT is not affected by these forced degradation procedures. The absence of additional peaks in the chromatogram of degradation study indicates non-interference of the common excipients used in the tablets and its degradants by peak purity. This demonstrates that the developed HPLC method is new, simple, linear, accurate, sensitive and reproducible, and can serve as stability indicating assay. The developed method can be easily used for the routine quality control of bulk and tablet forms.

## REFERENCES

1. Mullol, J., Bousquet, J., Bachert, C., Canonica, W.G., Gimenez-Arnau, A., Kowalski, M.L., et al., *Allergy*, 2008, vol. 63, p. 5.
2. Schoors, D.F., Smet, M.D., Reiss, T., Margolskee, D., Cheng, H., Larson, P., et al., *Br. J. Clin. Pharmacol.*, 1995, vol. 40, p. 277.
3. Nogueira, D.R., D'Avila, F.B., Rolim, C.M., and Dalmora, S.L.J., *Chromatographia*, 2007, vol. 66, p. 915.
4. Wen, J., Hong, Z., Wu, Y., Wei, H., Fan, G., and Wu, Y., *J. Pharm. Biomed. Anal.*, 2009, vol. 47, p. 347.
5. Shirkhedkar, A.A., Thorve, R.R., Fursule, R.A., and Surana, S.J., *Acta Chromatogr.*, 2008, vol. 20, p. 423.

6. Al-Rawithi, S., Al-Gazlan, U., Al-Ahmadi, W., Alshowaier, I., Yusuf, A., and Dale, A., *J. Chromatog.*, 2001, vol. 7543, p. 527.
7. Chester, J., Kitchen, I., Amy, Q., Wang, I., Musson, D.G., Yang, A.Y, et al., *J. Pharm. Biomed. Anal.*, 2003, vol. 31, p. 647.
8. Robert, P.A., Luka, P., Mulletta, W.M., and Elizabeth, K., *J. Chromatogr. B,* 2007, vol. 858, p. 282.
9. Chauhan, B., Rani, S., Nivsarkar, M., and Padh, H., *Ind. J. Pharm. Sci.*, 2006, vol. 68, p. 517.
10. Liu, L., Cheng, H., Zhao, J., and Roger, D., *J. Pharm. Biomed. Anal.*, 1997, vol. 15, p. 631.
11. Ochiai, H., Uchiyama, N., Takano, T., Hara, K.I., and Kamei, T., *J. Chromatogr. B*, 1998, vol. 2003, vol. 31, p. 359.
12. Radhakrishna, T., Narasaraju, A., Ramakrishna, M., and Satyanarayana, A., *J. Pharm. Biomed. Anal.*, 2003, vol. 31, p. 359.
13. Hong, T.H., Sharma, R., Sustanto, D., Maso, M.D., and Kwong, E., *J. Chromatogr. A*, 2007, vol. 1156, p. 149.
14. Hoang, T.H., Farkas, R., Wells, C., McClintock, S., and Maso, M.D., *J. Chromatogr. A*, 2002, vol. 968, p. 277.
15. See, M.M., Thompson, R., Grinberg, N., Perpall, H.J., Bicker, G., and Tway, P., *J. Liq. Chromatogr.*, 1995, vol. 18, p. 137.
16. Patel, P.G., Vaghela, V.M.L., Rathi, S.J., Rajgor, N.B., and Bhaskar, V.H., *J. Young. Pharm.*, 2009, vol. 1, no. 4, p. 354.