Validation of a High-Performance Liquid Chromatography Method for the Assay of and Determination of Related Organic Impurities in Nevirapine Drug Substance

Q. Chan Li[1,*], **T. Tougas**[1], **K. Cohen**[1], **R. Lee**[1], **P. Meagan**[1], **M. Corson**[2], and **T. Muchnick**[3]

[1]Analytical Sciences Department, Research and Development Center, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT 06877; [2]Pharmaceutics Department, Research and Development Center, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT 06877; and [3]Purdue Pharma, L.P., 444 Saw Mill River Rd., Ardsley, NY 10502

Abstract

Nevirapine (Viramune™), a dipyridiodiazepinone, is a potent and highly specific nonnucleoside inhibitor of HIV-1 reverse transcriptase. This paper describes the validation of a specific, sensitive, and stability-indicating high-performance liquid chromatography method for the assay and determination of related organic impurities in nevirapine drug substance. This method uses a Supelcosil LC-ABZ column, a mobile phase of 20:80 (v/v) acetonitrile–25mM $NH_4H_2PO_4$ (pH 5.0), and ultraviolet detection at a wavelength of 220 nm. This method was validated for specificity, linearity, accuracy, repeatability, detection limit, quantitation limit, stability of analyte solutions, robustness, and intermediate precision. Nevirapine is completely separated from all impurities. The method is shown to be linear with coefficients of determination r^2 greater than 0.999. Average accuracy is 100.4% with a relative standard deviation of 0.7% for the assay. Accuracy ranges from 100.1 to 102.6% for related organic impurities. Repeatability is good, with relative standard deviations not more than 1.4%. The detection limit and the quantitation limit are determined to be 0.001 and 0.003%, respectively. Relative response factors of known organic impurities are determined, permitting the use of nevirapine at the 0.1% level as an external standard for the quantitation of these impurities. Analyte solutions are shown to be stable for at least 2 days at ambient temperature. The method is validated as robust, and intermediate precision is high. A system suitability test is developed and validated, and requirements are set.

Introduction

Nevirapine (Viramune™), a dipyridiodiazepinone, is a member of a class of antiretroviral compounds known as nonnucleoside reverse transcriptase inhibitors. Nevirapine is a potent and selective noncompetitive inhibitor of the reverse transcriptase of human immunodeficiency virus type-1 (HIV-1). In completed clinical trials, nevirapine has demonstrated antiretroviral activity both as monotherapy and in combination with nucleoside analogues including zidovudine or the combination zidovudine–didanosine. The antiviral effect has been profound and sustained (1). Viramune was recently featured in *Health Magazine* (2) as one of the top ten medical advances of 1999 and on CNN's list (December 29, 1999) of the top 10 health improvement stories of 1999. Viramune was lauded as the ninth greatest health advance in 1999 based on its ability to reduce the transmission of HIV from mother to infant. In addition, nevirapine is more affordable and practical than any other drug examined to date. The *Health Magazine* (2) article reports, "The new drug [nevirapine] is as cheap as it is effective, costing about one-senventieth as much as a short course of AZT. The Ugandan government is working with the manufacturer to make the drug widely available in that country and other Afriacn nations may follow. If so, researchers estimate 400,000 infants could be spared from HIV each year."

Three papers have been published on chromatographic methods that study different aspects of nevirapine. Dinallo et al. (3) reported the characterization of synthetic byproducts by B/E linked scanning and high-resolution thermospray mass spectrometry. Cohen et al. (4) and Palladino et al. (5) separately reported the studies of the binding environment of nevirapine to reverse transcriptase of HIV-1 by high-performance liquid chromatography (HPLC) and photoaffinity crosslinking.

The present paper is the first to describe the validation of a specific, sensitive, and stability-indicating HPLC method for the assay and determination of related organic impurities in nevirapine drug substance. The validation generally complies with the International Conference on Harmonisation (ICH) guidelines on the impurities in new drug substances (6) and

* Author to whom correspondence should be addresed.

the validation of analytical methods: definition and terminology (7) and methodology (8).

Experimental

Chemicals and reagents

OmniSolv HPLC-grade acetonitrile and methanol were purchased from EM Science (Gibbstown, NJ). Water was purified from a Milli-Q (Milford, MA) purification system. HPLC-grade $NH_4H_2PO_4$ was purchased from Fisher Scientific (Fair Lawn, NJ). All drug and drug–related substances were prepared and characterized by Boehringer Ingelheim Pharmaceuticals (Ridgefield, CT).

Apparatus

Three HPLC systems were mainly used in this work. System 1, which was primarily used, was purchased from Waters Corporation (Milford, MA) and consisted of a model 616 four-solvent delivery system and controller, a 717 WISP sample injector, a model 996 photodiode array detector (PDA), and a 486 NEC (Melville, NY) computer. PDA data were acquired and analyzed on the computer using Waters Millennium 2010 Chromatography Manager (version 2.10). HPLC System 2 was an HP1090 LC (Hewlett-Packard, Wilmington, DE) integrated with a series II diode array detector (DAD). System 3 consisted of a PE 250 binary pump (Perkin-Elmer, Norwalk, CT) and a Kratos (Chestnut Ridge, NY) 757 Spectroflow variable wavelength detector. An Orion (Beverly, MA) model 520A pH meter was used to measure the pH of phosphate aqueous solution.

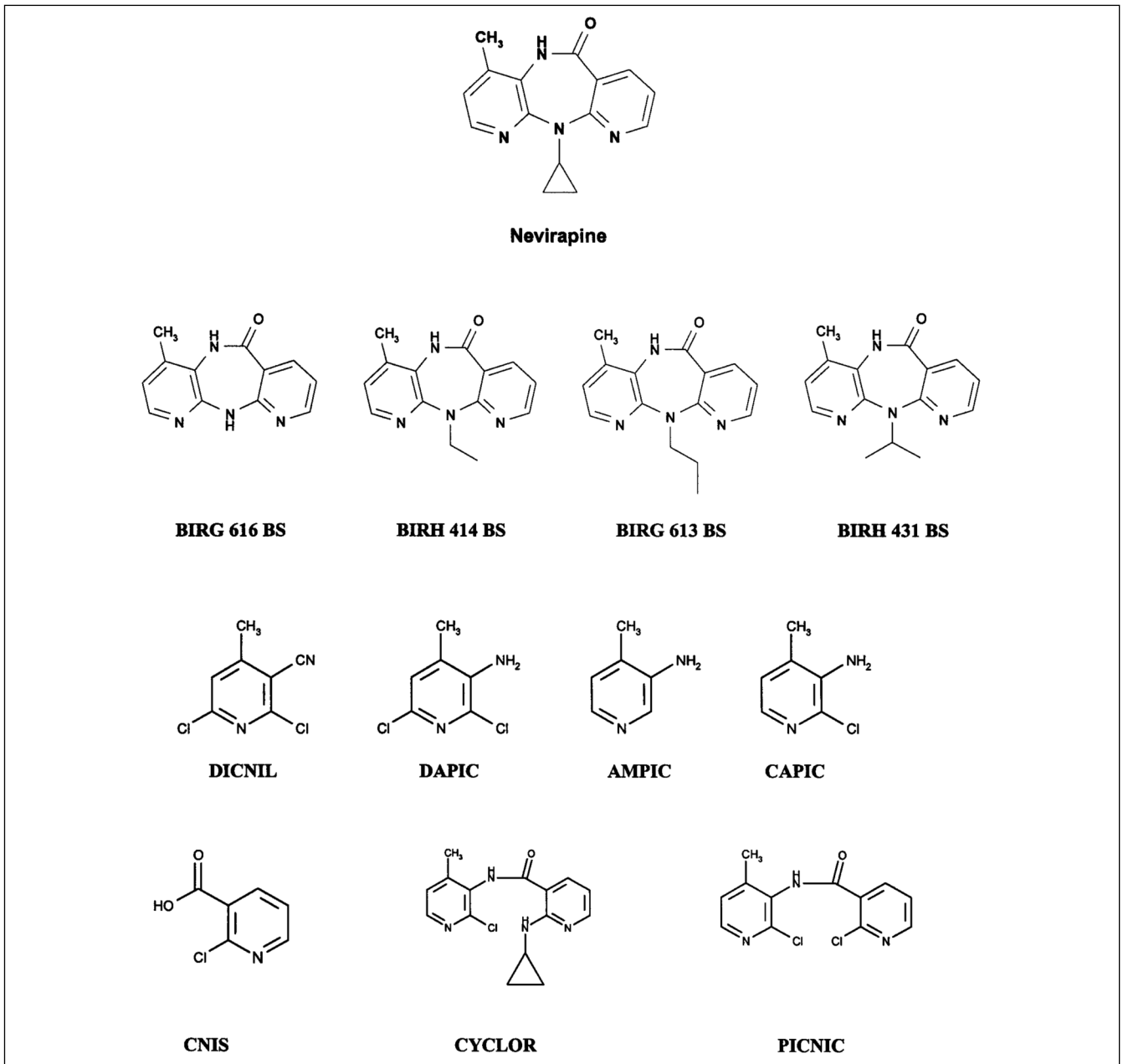

Figure 1. Chemical structures of nevirapine and related substances from syntheses.

Chromatographic conditions

The column was Supelcosil LC-ABZ (150 × 4.6 mm, 5-µm particle size, Supelco, Bellefonte, PA). The column oven temperature was 35°C. The mobile phase was 20:80 (v/v) acetonitrile–25mM $NH_4H_2PO_4$ (pH 5.0), and the flow rate was 1 mL/min. The injection volume for the assay and system suitability test was 25 µL, and that for the determination of related organic impurities was 50 µL. The run time for the assay and system suitability test was 10 min, and that for the determination of related organic impurities was 48 min. All chromatograms were obtained with ultraviolet (UV) detection at a wavelength of 220 nm.

Solution preparation

Nevirapine stock solutions (0.24 mg/mL)

Approximately 24 mg of Nevirapine (structure shown in Figure 1) reference standard was transferred into a 100-mL volumetric flask. Then, 4 mL of acetonitrile and approximately 80 mL of mobile phase were added. The flask was sonicated until all material was dissolved (as indicated by no visible powder at the bottom of the flask). The solution (100% level) was cooled to ambient temperature and then diluted to volume with mobile phase.

BIRH 414 BS stock solution (0.24 mg/mL)

Approximately 6 mg of BIRH 414 BS (sturcture shown in Figure 1) reference material was transferred into a 25-mL volumetric flask. Then, 5 mL of acetonitrile and approximately 15 mL of mobile phase were added. The flask was sonicated (typically 15–25 min) until all material was dissolved (as indicated by no visible powder at the bottom of the flask). The solution was cooled to ambient temperature and then diluted to volume with mobile phase.

BIRG 616 BS stock solution (0.06 mg/mL)

Approximately 6 mg of BIRG 616 BS (structure shown in Figure 1) reference material was transferred into a 100-mL volumetric flask. Then, 25 mL of acetonitrile and approximately 55 mL of mobile phase were added. The flask was sonicated (typically 30–35 min) until all material was dissolved (as indicated by no visible powder at the bottom of the flask). The solution was cooled to ambient temperature and then diluted to volume with mobile phase.

The following solutions were prepared from the above stock solutions with appropriate dilution, and the mobile phase was used as the diluent.

System suitability test solution

The system suitability test solution contained approximately 14.4 µg/mL of BIRG 616BS, 28.8 µg/mL of nevirapine, and 28.8 µg/mL of BIRH 414 BS.

Standard solutions

For the assay, the standard solution contained approximately 28.8 µg/mL of nevirapine standard. For the determination of related organic impurities, the standard solution

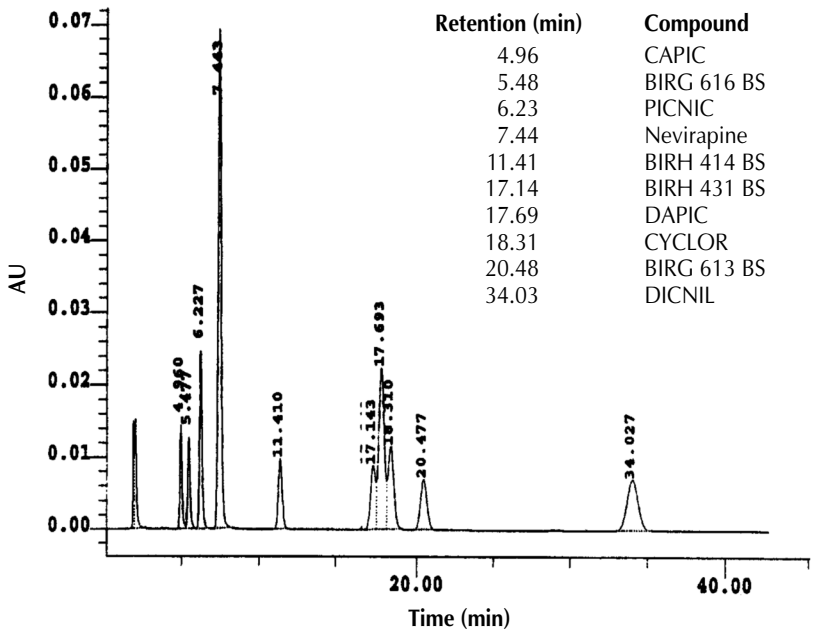

Figure 2. Selectivity chromatogram.

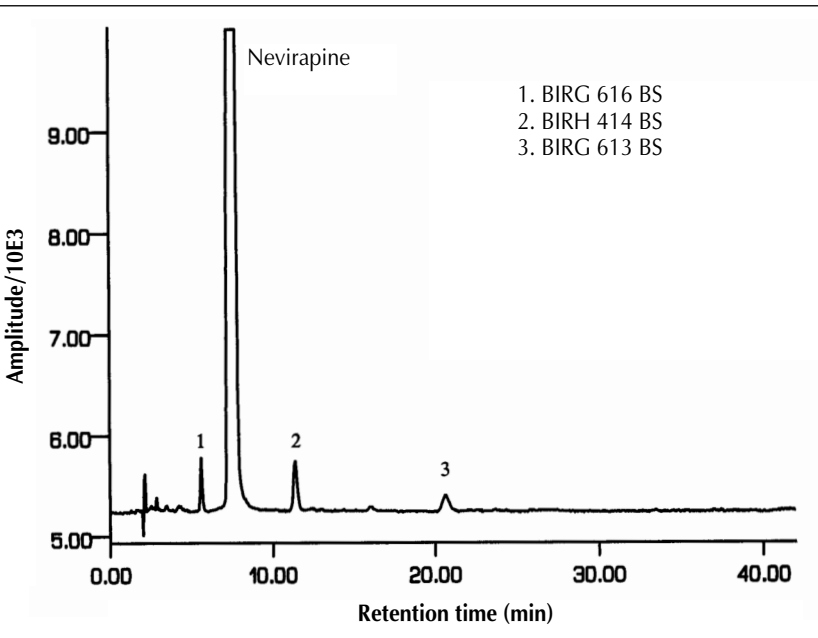

Figure 3. A typical nevirapine sample chromatogram for chromatographic purity.

contained approximately 0.24 µg/mL of nevirapine (0.1% level).

Sample preparation

For the assay, a sample contained approximately 28.8 µg/mL of nevirapine. For the determination of related organic impurities, a sample contained approximately 0.24 mg/mL nevirapine, which was prepared in a manner similar to that for the nevirapine standard stock.

Results and Discussion

This method was validated for specificity, linearity, accuracy, repeatability, detection limit, quantitation limit, stability of analyte solutions, robustness, and intermediate precision.

Specificity

Selectivity

Figure 1 shows the chemical structures of nevirapine and related substances including starting materials, intermediates, and synthetic byproducts. Figure 2 shows a typical selectivity chromatogram. Nevirapine was completely separated from all other compounds. CAPIC, BIRG 616 BS, PICNIC, BIRH 414 BS, BIRG 613 BS, and DICNIL were all baseline resolved from adjacent peaks. Though BIRH 431 BS, DAPIC, and CYCLOR were partially coeluted, their UV spectra are markedly different, allowing reliable identification. AMPIC and CNIS, the precursors preceding the final step of synthesis, partially coeluted and were near the solvent front. These two water-soluble precursors were easily removed in the process of purification and have never been detected in any lot of nevirapine drug substance. In fact, only three process impurities (BIRG 616 BS, BIRH 414 BS, and BIRG 613 BS) are commonly present at < 0.1% in nevirapine drug substance at release (Figure 3). In addition, long-term (3 years) stability studies show that nevirapine does not decompose under normal storage conditions. Therefore, there are no degradation products that need to be monitored.

Stress studies

To demonstrate the stability-indicating character of the method, nevirapine samples were subjected to stress by acid, base, hydrogen peroxide, UV light, and heat/humidity. The stressed samples were assayed to determine the percentage of remaining nevirapine. The homogeneity and integrity of the nevirapine peak in each stressed sample were examined by overlaying UV spectra from 7 positions of the peak and comparing with that of the nevirapine standard and by purity testing and spectral contrasting techniques using Waters Millennium 2010 Chromatography Manager (version 2.10) and reference 9. Table I summarizes the results along with the stress conditions (spectral overlays and purity testing results are not shown).

Under each stress condition, the nevirapine peak was shown to be spectrally pure, because the purity angle was less than the purity threshold. Furthermore, the integrity of the nevirapine peak was intact, because the spectrum of the peak at its apex matched that of the nevirapine standard, and the matching angle was less than the matching threshold (9). The results in Table I establish that the method is stability-indicating.

Table I. Stress Studies

Stress conditions*	Duration	Peak integrity	% Nevirapine remaining
1N HCl (RT)	31 days	pure†	29.64
1N NaOH (RT)	31 days	pure†	81.50
3% H_2O_2 (RT)	31 days	pure†	71.75
Light (600 foot candles)	31 days	pure†	99.99
40°C/75%RH	6 months	pure†	99.81

* RT = ambient temperature.
† Purity angle was less than purity threshold, confirming peak purity.

Table II. Assay Linearity of Nevirapine

Weighing 1			Weighing 2		
% Assay level	Nevirapine injected (µg)	Peak area	% Assay level	Nevirapine injected (µg)	Peak area
47.1	0.3388	1053750	50.4	0.3626	1136931
70.6	0.5082	1579270	75.5	0.5439	1701925
94.1	0.6776	2089467	100.7	0.7252	2250666
117.6	0.8470	2625989	125.9	0.9065	2831016
141.1	1.0160	3143074	151.1	1.0878	3415734
Slope	***y*-Intercept**	r^2	**Slope**	***y*-Intercept**	r^2
3086089	7423	0.99996	3136623	–7424	0.9998

Assay validation

Linearity (range)

The linearity of the area responses of nevirapine was validated for two separate weighings at concentrations ranging from 47 to 151% of the assay level. A linear regression analysis was performed for each weighing set. The coefficients of determination (r^2) were all greater than 0.999, indicating a high degree of linearity. Results for these experiments are summarized in Table II and Figure 4.

Accuracy (recovery)

Aliquots of mobile phase were spiked with nevirapine at five evenly spaced levels corresponding to 50–153% of the assay level. These five levels were produced from five

separate weighings. Each level was injected in duplicate, and area responses were compared to those of two external standards. The average accuracy was 100.4% with a relative standard deviation (RSD) of 0.7%, indicating very good accuracy. The results are summarized in Table III.

Repeatability

Assay repeatability was validated by measuring the area response factors of five separate sample preparations of nevirapine of the same lot. Each preparation was injected in duplicate. The average response factor was 4.3792E-07 µg/area with an RSD of 0.36%, indicating a high degree of repeatability. Results for these experiments are summarized in Table III.

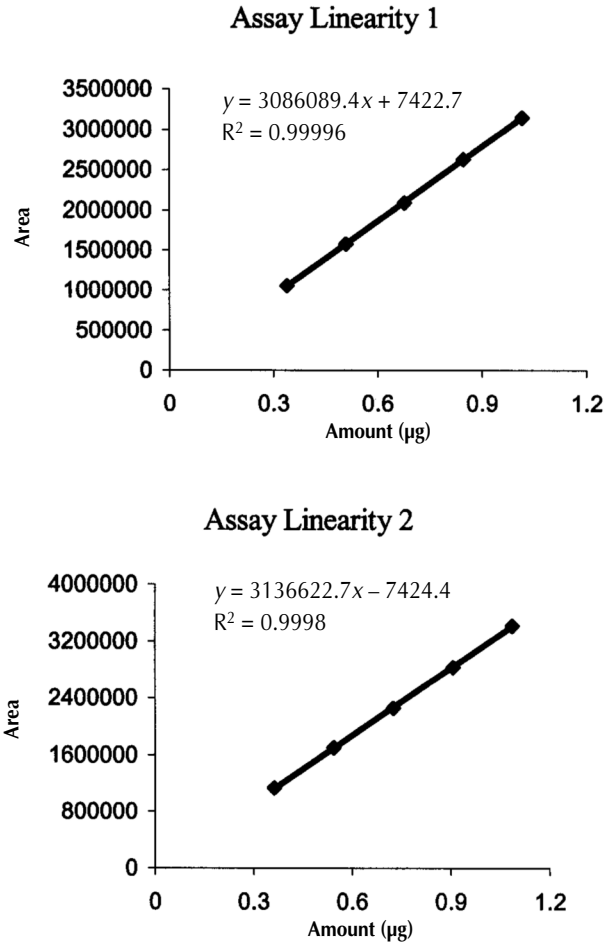

Figure 4. Linear regression plots for the linearity validation of the assay of nevirapine.

Validation for the determination of related organic impurities

Each parameter in this section was validated for nevirapine and three observed impurities (BIRG 616 BS, BIRH 414 BS, and BIRG 613 BS) unless otherwise stated. The range for BIRG 616 BS was 0.1–0.3% (w/w), and the range for both BIRH 414 BS and BIRG 613 BS was 0.15–0.45% (w/w).

Linearity (range) and relative response factors

Linearity was validated by measuring area responses for each of the four compounds at the concentration ranges previously mentioned. For each compound, two separate sample preparations were made, the same serial dilutions were made, and each preparation was injected in duplicate. A linear regression analysis was performed. All the coefficients of determination r^2 were greater than 0.999, indicating a high degree of linearity. The data from these experiments are summarized in Table IV.

Response factors were calculated for nevirapine, BIRG 616 BS, BIRH 414 BS, and BIRG 613 BS from the linearity data, and relative response factors (versus nevirapine) for the three impurities were also calculated. Results are summarized in Table IV. In practice, impurities are quantitated relative to a nevirapine standard, and the levels of the three known impurities are then corrected with respective relative response factors.

Repeatability

Repeatability was validated for each of the four compounds by measuring response factors at three concentration levels, which were approximately 0.1, 0.2, and 0.3% for BIRG 616 BS and approximately 0.15, 0.30, and 0.45% for BIRH 414 BS and BIRG 613 BS. Each preparation was injected in duplicate. An RSD was determined from these six injections for each compound. The results are

Table III. Assay Accuracy and Repeatability

Accuracy		Repeatability		
% Assay level	% Accuracy	Nevirapine injected (µg)	Area	Response factor (µg/area)
50.4	100.0, 99.9	0.7296	1675709, 1673073	4.3540E-07, 4.3608E-07
77.5	101.5, 101.6	0.6999	1599560, 1596161	4.3756E-07, 4.3849E-07
104.9	100.7, 100.7	0.7230	1650644, 1649480	4.3801E-07, 4.3832E-07
129.3	99.6, 99.7	0.7080	1609922, 1606466	4.3977E-07, 4.4072E-07
152.6	100.2, 100.2	0.7149	1635455, 1632432	4.3713E-07, 4.3794E-07
Average	100.4		Average	4.3794E-07
RSD (%)	0.7		RSD (%)	0.36

Table IV. Linearity and Relative Response Factors for Related Organic Impurities

Sample set 1/system 1						Sample set 2/system 2					
% Concentration*	Average area	Slope area/ng	*y*-Intercept	r^2	RRF	% Concentration*	Average area	Slope area/ng	*y*-Intercept	r^2	RRF
Nevirapine											
0.14	83711					0.15	15406				
0.29	166918	4761.3	2253	0.99991	1.00	0.29	30292	844.4	694.2	0.99994	1.00
0.43	247501					0.44	44875				
BIRG 616 BS											
0.09	69105					0.09	12846				
0.18	134324	6019	4960	0.99995	1.30	0.19	25171	1100	415	0.9999997	1.29
0.27	197906					0.28	37599				
BIRH 414 BS											
0.15	87986					0.15	15406				
0.30	176549	4829	1447	0.99994	1.01	0.29	30593	862	240	0.9999996	1.00
0.45	262801					0.44	45748				
BIRG 613 BS											
0.15	83185					0.15	15317				
0.30	167362	4650	890	0.99990	0.97	0.30	30246	822	556	0.99998	0.97
0.45	248916					0.45	44923				

* Concentration level (or % impurity level) was relative to nevirapine at the 100% level.

Table V. Repeatability for Related Organic Impurities

Nevirapine		BIRG 616 BS		BIRH 414 BS		BIRG 613 BS	
% Concentration level*	RF $\times 10^3$ (ng/area)	% Concentration level*	RF $\times 10^4$ (ng/area)	% Concentration level*	RF $\times 10^3$ (ng/area)	% Concentration level*	RF $\times 10^3$ (ng/area)
0.15	1.123	0.09	8.764	0.15	1.139	0.15	1.181
1.143		8.780		1.147		1.171	
0.29	1.152	0.19	8.969	0.29	1.157	0.30	1.185
1.153		8.937		1.144		1.198	
0.44	1.166	0.28	8.985	0.44	1.162	0.45	1.205
1.168		9.001		1.147		1.201	
Average	1.151E-3	**Average**	8.905E-4	**Average**	1.149E-3	**Average**	1.190E-3
RSD (%)	1.4	**RSD (%)**	1.2	**RSD (%)**	0.8	**RSD (%)**	1.1

* Concentration level (or % impurity level) was relative to nevirapine at the 100% level.
† RF = response factor.

Table VI. Accuracy for Related Organic Impurities

BIRG 616 BS		BIRH 414 BS		BIRG 613 BS	
% Concentration*	% Accuracy	% Concentration*	% Accuracy	% Concentration*	% Accuracy
0.05	98.6	0.05	97.2	0.05	102.9
	97.0		95.5		100.2
	98.0		100.9		104.7
0.20	101.1	0.20	101.4	0.20	101.6
	100.0		101.1		101.5
	101.9		99.8		102.5
0.25	101.7	0.25	103.8	0.25	103.3
	101.4		103.6		104.1
	101.3		103.4		102.5
Average	100.1	**Average**	100.7	**Average**	102.6
RSD (%)	1.8	**RSD (%)**	2.9	**RSD (%)**	1.4

* Concentration level (or % impurity level) was relative nevirapine at the 100% level.

summarized in Table V. All RSDs were no greater than 1.4%, showing a high degree of repeatability.

Accuracy

Accuracy was validated by spiking drug substance sample preparations with each of the three potential impurities at 3 levels ranging from 0.05 to 0.25% and injecting each preparation in duplicate. For each impurity and each injection, the area response of the matrix interference was subtracted, and then the corrected area response was compared to that of two corresponding external standards. Results are summarized in Table VI. Recoveries were from 100.1 to 102.6%, indicating no loss for any of these three impurities.

Detection and quantitation limits

The detection limit (DL) and quantitation limit (QL) for nevirapine were determined on three columns: a new column (new), a column used throughout development (middle-aged), and a column that had been extensively used with other mobile phases (old). DL and QL were calculated as follows:

$$\text{DL or QL} = kC(S_B/S)$$

where k = 3 for DL and 10 for QL, C is the level of nevirapine expressed as a percent (e.g., 0.01%), and S_B is the standard deviation of area responses. In practice, S_B was determined by injecting (n = 5) a 0.01% nevirapine solution. S is the average area response.

Table VII presents the results of the measurements. DL was determined to be 0.001% (w/w) on all three columns. QL was found to be 0.002% (w/w) on the new and middle-aged columns and 0.003% (w/w) on the old column. The results indicate that the method was very sensitive.

Stability of analyte solutions

The stability of typical standard preparations for both the assay and determination of related organic impurities was monitored by measuring area responses of injections made over a period of 2 days for the two standards. The relationships between area response and time are plotted in Figure 5. The area responses exhibited no trending up or down. An RSD for the area responses of the 13 injections of the chromatographic purity standard was 0.44%, and an RSD for the area responses of the 12 injections of the assay standard was 0.61%. The results indicate that the

Table VII. Measurement of DL and QL

Column Age	Area	DL & QL calculations
New	6210	C = 0.01%
	6353	DL = 3C(S_B/S)
	6130	DL = 10C(S_B/S)
	6131	
	6077	
	S = 6180	DL = 3 × 0.01% × (108/6180) = 0.001%
	S_B = 108	QL = 10 × 0.01% × (108/6180) = 0.002%
Middle-aged	6322	
	6153	
	6312	
	6181	
	6488	
	S = 6291	DL = 3 × 0.01% × (134/6291) = 0.001%
	S_B = 134	QL = 10 × 0.01% × (134/6291) = 0.002%
Old	8220	
	8117	
	8329	
	8394	
	8696	
	S = 8351	DL = 3 × 0.01% × (220/8351) = 0.001%
	S_B = 220	QL = 10 × 0.01% × (220/8351) = 0.003%

Table VIII. Influences of Variations in Chromatographic Conditions on System Suitability Test Parameters

Variation	Rs, 616/NVP* > 5.0	Rs, NVP/414* > 7.4	Assay nevirapine (%)
No variation‡	6.6	10.6	99.1
Phosphate solution pH			
4.9 (–)	6.8	10.6	99.1
5.1 (+)	6.8	10.6	99.0
Phosphate weight (g)			
2.86 (–)	6.4	10.4	98.9
2.90 (+)	6.5	10.5	98.9
Acetonitrile volume (mL)			
190 (–)	7.0	10.8	99.0
205 (+)	6.6	10.5	98.8
Column temperature (°C)			
32 (–)	6.3	10.5	99.1
38 (+)	6.9	10.7	99.1
Flow rate (mL/min)			
0.9 (–)	6.7	10.7	99.1
1.1 (+)	6.5	10.3	98.9
Column lot			
1	7.2	10.8	99.1
2	6.3	10.6	99.0
3	6.3	10.4	99.1
4	5.8	9.8	98.9
5	6.0	10.1	99.0
No variation‡	6.6	10.5	99.1

* 616 = BIRG 616 BS, NVP = nevirapine, 414 = BIRH 414 BS.
† System suitability requirements.
‡ No variation consisted of a phosphate solution of pH 5.0, phosphate weight of 2.88 g, acetonitrile volume of 200 mL, column temperature of 35°C, and flow rate of 1.0 mL/min.

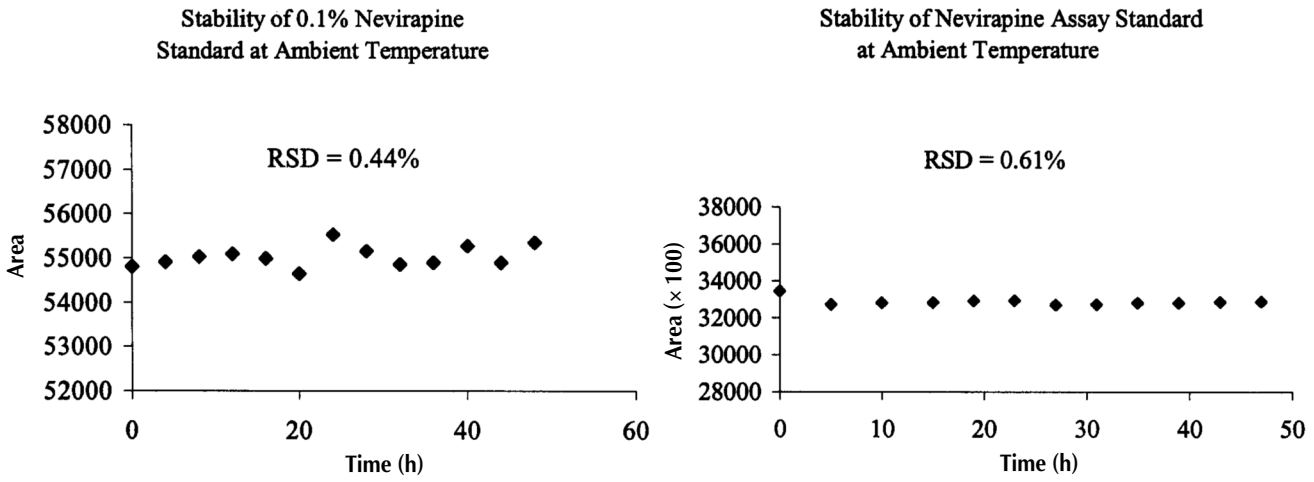

Figure 5. Ambient-temperature stability of the 0.1% nevirapine standard and assay standard solutions.

Table IX. Effect of Column Age on System Performance Criteria

	Resolution		
	New	Middle-aged	Old
Compound pair			
BIRG 616 BS–nevirapine	6.2	7.2	5.3
Nevirapine–BIRH 414 BS	9.8	10.8	8.7
Nevirapine peak			
Tailing factor	1.1	1.2	1.1
Theoretical plates	9973	10097	9035

Table X. Analyst, System, Column, Column Batch, And Day Precision*

	Retention time (min)					
	A1/S1		A2/S2		A3/S3	
Compound	C1/B1	C2/B2	C1/B1	C2/B2	C1/B1	C2/B2
BIRG 616 BS	5.4	5.4	5.8	5.8	5.6	5.5
Nevirapine	7.2	7.2	7.8	7.7	7.4	7.3
BIRH 414 BS	11.3	11.2	12.1	11.9	11.4	11.3
BIRG 613 BS	20.6	20.4	22.1	21.7	20.6	20.4
	Resolution					
	A1/S1		A2/S2		A3/S3	
Compound Pair	C1/B1	C2/B2	C1/B1	C2/B2	C1/B1	C2/B2
BIRG 616 BS–nevirapine	5.8	6.0	5.7	5.8	5.6	5.8
Nevirapine–BIRH 414 BS	9.8	10.0	10.5	10.8	9.4	9.8

* A = analyst, S = HPLC system, C = column, and B = column batch.

nevirapine solutions were stable for 2 days at ambient temperature.

Robustness

A robustness study was performed by making small but deliberate variations in the method parameters. The effects of the variations in the chromatographic conditions on system suitability test parameters and on the assay of the nevirapine drug substance were studied, and the results are presented in Table VIII. The results show that under all the variations, the requirements of the system suitability test are met, and the assay values of the nevirapine drug substance are consistent. The method is robust.

Intermediate precision

The intermediate precision was evaluated by examining the effect of column age, different columns, analysts, systems, and days on chromatographic performance. A test solution composed of Nevirapine, BIRG 616 BS, BIRH 414 BS, and BIRG 613 BS was used to evaluate intermediate precision. The following results from the analysis of this test solution show that the method has a high degree of intermediate precision.

Column age

The effect of column age on several chromatographic parameters was evaluated. Three columns that were new, middle-aged, and old (described in a previous section) were examined. The measured parameters were resolution for two

pairs (BIRG 616 BS and nevirapine, nevirapine and BIRH 414 BS), tailing factor (T_f), and theoretical plate number (N) for nevirapine. The results are summarized in Table IX. All three columns provided baseline separation for the four compounds. The resolution between BIRG 616 BS/nevirapine and between nevirapine/BIRH 414 BS were all respectively greater than 5.0 and 7.4, the values specified in the system suitability requirements (in the following section). All three columns also gave acceptable tailing factors and theoretical plate numbers.

Different analysts, systems, columns, column batches, and days

The precision for different analysts, systems, columns, column batches, and days was validated. Table X summarizes the results representing three analysts (A1, A2, and A3) working autonomously on 3 days with three HPLC systems (S1, S2, and S3) from three manufacturers and two columns (C1 and C2) taken from two batches (B1 and B2). The results in Table X show that the intermediate precision is high.

System suitability test

The results in Tables VIII–X demonstrate that the method is robust and precise, because nevirapine and the three actual impurities BIRG 616 BS, BIRH 414 BS, and BIRG 613 BS are well separated in all studied cases. Based on these results, a system suitability test (SST) to ensure overall system performance was developed for the method. The SST solution was designed to contain nevirapine, BIRG 616 BS, and BIRH 414 BS. BIRG 616 BS is a commonly observed impurity and elutes before nevirapine, whereas BIRH 414 BS is another commonly observed impurity and elutes after nevirapine. Therefore, two parameters, resolution (Rs_1) between BIRG 616 BS and nevirapine and resolution (Rs_2) between nevirapine and BIRH 414 BS, provide rigorous analytical control to ensure the proper performance of an HPLC system. An HPLC system is deemed suitable if Rs_1 is not less than 5.0 and Rs_2 not less than 7.4.

Conclusion

An HPLC method was developed and validated for the assay and determination of related organic impurities in nevirapine drug substance. This method is specific, sensitive, robust, precise, and capable of accurately assaying nevirapine and determining impurities in this drug substance.

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