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Determination of the endothelin receptor antagonist ABT-627 and related substances by high performance liquid chromatography

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Abstract

The determination of the endothelin (ET) antagonist receptor ABT-627 (I) and related substances is performed by HPLC. I is determined in bulk drug substance and drug formulation using isocratic conditions and an Inertsil ODS-2 column. The determination is stability indicating and detector response is linear from 24 to 118 μg ml^{-1} (33–164% of assay level). Intermediate precision for the determination ranged from ±0.60 to ±1.9% RSD. The measurement is accurate, with quantitative recovery of I from the formulation placebo. Related substances in I and formulated I are determined using the same chromatographic conditions, with a gradient elution profile to elute impurities having varying relative polarities. The detector response for related substances determination is linear for I from 0.60 to 17.8 μg ml^{-1} (0.05–1.5% of assay level) with the limit of detection and quantitation estimated at 0.01 and 0.05%, respectively. Comparable precision was obtained in drug substance and drug formulation (RSD values ±3.7 to ±12% and ±5.5 to 16.9%, respectively for impurities ranging from 0.05 to 0.30%). The quantitated impurities agreed well for the same lot of I when assayed as a bulk substance and after the formulation into a drug product. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Endothelin antagonist; HPLC; Pharmaceutical analysis

1. Introduction

A number of potent nonpeptide endothelin (ET) receptor antagonists are under development for the potential treatment of several diseases. These agents provide varying degrees of selectivity as receptor antagonists for the three isoforms of ET, a highly active and long-lasting vasoconstrictor [1,2]. ABT-627 (I), Fig. 1, is an investigational drug synthesized as the 2R, 3S, 4S enantiomer. The chemical name of ABT-627 is [2R-(2α,3β,4α)]-4-(1,3-benzodioxol-5-yl)-1-[2-(dibutylamino)-2-oxoethyl]-2-(4-methoxyphenyl)-3-pyrrolidinecarboxylic acid, monohydrochloride. The racemate of I was previously reported as one of a

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series of synthesized 2,4-diarylpyrrolidine-3-carboxylic acids and was characterized as a potent and selective ET receptor antagonist [3]. The relative potency of the racemic compound was evaluated by comparing four other ET receptor antagonists in a series of binding studies [4].

A significant part of the successful development of a new drug substance is the ability to reliably determine the drug and to evaluate its purity prior to and after formulation in the early stages of development. High performance liquid chromatography (HPLC) is used extensively for this purpose. The ET receptor antagonist Bosenton (RO 47-0203) has been determined by chromatography with tandem mass spectrometry in plasma [5]. HPLC has also been used for the determination of the ET receptor antagonist Butenolide (PD 156707) in plasma [6]. In this work we report the determination of related substances in the drug substance and in a formulation of I using HPLC.

2. Experimental

2.1. Apparatus

The typical HPLC system consisted of a Model P-2000 binary pump and Model 100 variable wavelength detector (Thermo Separation Products, Santa Clara, CA) with a Model C-R4AX data station and a Model SIL-10A autosampler (Shimadzu, Kyoto, Japan). Chromatographic separations were performed on an Inertsil ODS-2 column (5 μm) (Metachem or Phenomenex, Torrance, CA). Related substances were determined on a column measuring 25 cm × 4.6 mm I.D. whereas the potency determinations used a 15 cm × 4.6 mm column. The HPLC eluent was filtered through 0.45 μm nylon membranes (Alltech Associates, Deerfield, IL).

2.2. Reagents

Acetonitrile was HPLC grade from EM Science (Gibbstown, NJ). Potassium phosphate, monobasic and orthophosphoric acid (85%) were reagent grade from J.T. Baker (Phillipsburg, NJ). *p*-Diethoxybenzene was reagent grade from Pfaltz and Bauer (Waterbury, CT). Compound I and related substances were manufactured at Abbott Laboratories (North Chicago, IL).

2.3. Chromatographic conditions

A 0.01 M potassium phosphate buffer (0.05 M for assay) was prepared in water and the pH was adjusted to 2.9 ± 0.1 (related substances) or 3.0 ± 0.1 (assay) using ortho-phosphoric acid. The flow rate was 1 ml min^{-1} and the injection volume was 20 μl. Detection was at 234 nm at 0.10 AUFS, attenuated at the data station. The following gradient elution profile was used for determining related substances.

Time (min)	% 0.01 M Phosphate buffer	CH_3CN
0 (Initial)	53	47
15 (Initial)	53	47
25	20	80
50	20	80
55	53	47
70	53	47

The initial conditions were used for the determination of I. Temperature was ambient for all separations.

2.4. Assay procedure

2.4.1. Determination of related substances

For the drug substance, I was prepared in a diluent which is a 1:1 mixture of 0.01 M phos-

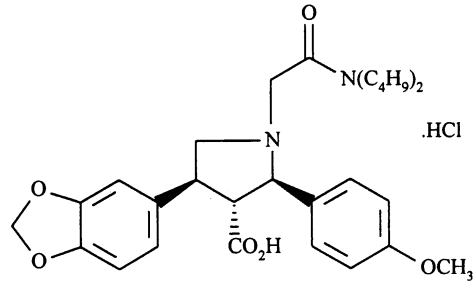

Fig. 1. Structure of ABT-627.

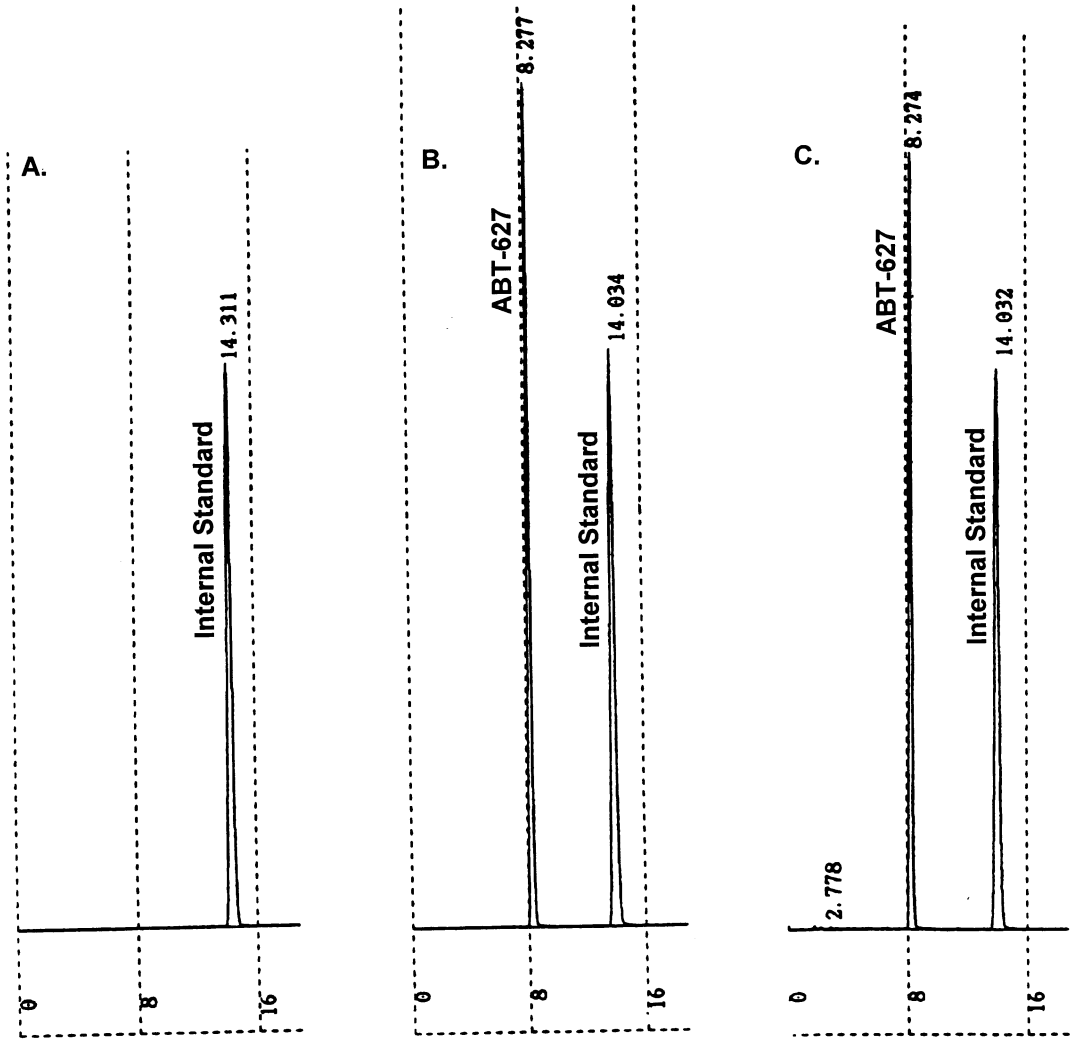

Fig. 2. Typical chromatograms for the determination of I in the drug product. A, internal standard; B, standard preparation; C, sample preparation from 200 mg capsules.

phate buffer and acetonitrile at a concentration of ~ 1.2 mg ml^{-1}. For the drug product, the capsule fill composite of I is extracted by shaking with the diluent for 15 min and the volume is adjusted to yield a solution containing ~ 1.2 mg ml^{-1}. The standard preparation is I prepared in diluent at ~ 6 μg ml^{-1} (0.5% of the sample preparations). The chromatographic conditions were initially adjusted to elute I in the standard preparation at $>25\%$ full scale response at a retention time of 10.5–13.5 minutes. Replicate injections were made for the standard ($N = 5$, RSD $\leq 5\%$) and the mean peak response was used. A tailing factor ($T = W_{0.05}/2f$, USP 23) of ≤ 1.8 was also used as a measure of system suitability. Individual peaks for the related substances detected in the sample preparation were quantitated by the external standard method and tabulated by relative retention time of the peak to the parent in the sample chromatogram.

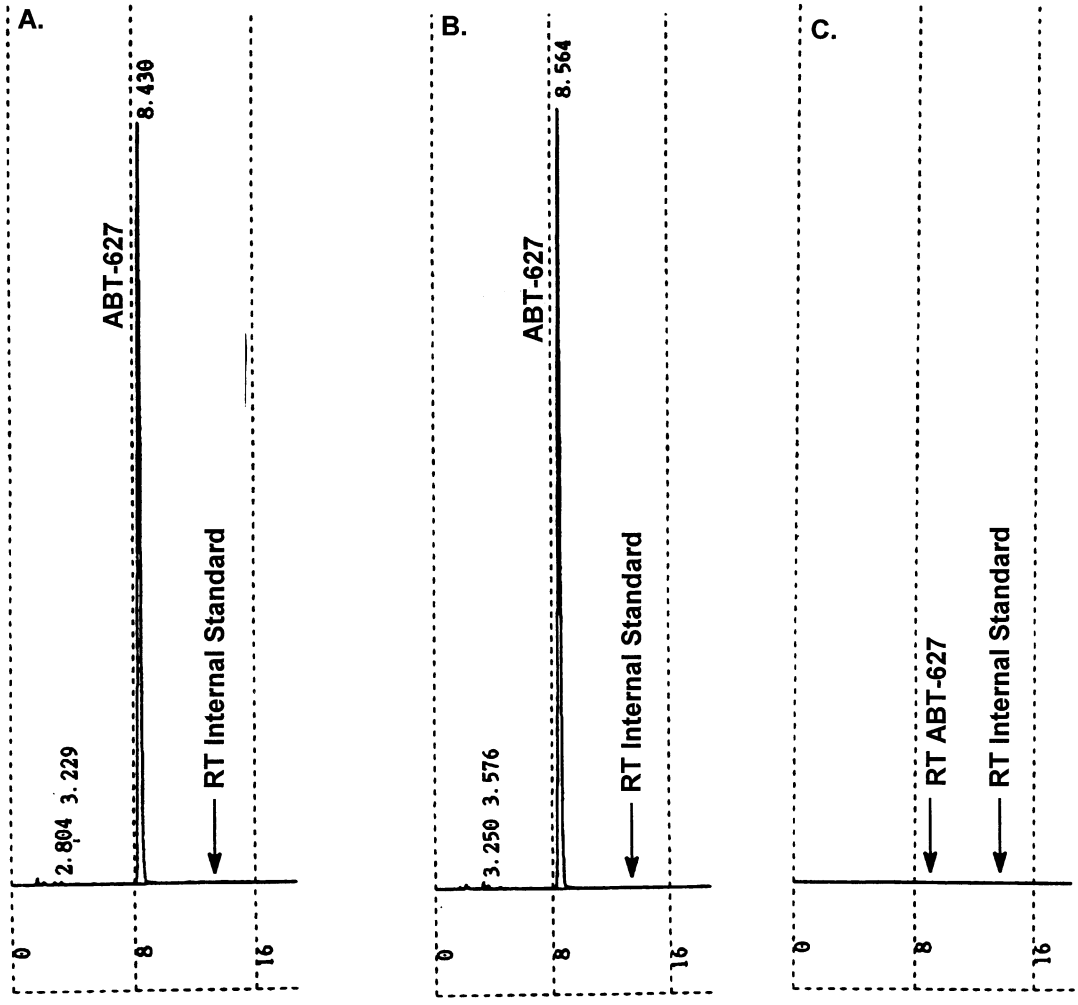

Fig. 3. Chromatograms for sample fill of the drug product after stressing. A, refluxed in H_2O for 1 h; B, heated at 105°C for 48 h; C, placebo heated at 105°C for 48 h.

2.4.2. Strength determination of I

The sample preparations described for the determination of related substances were diluted to 72 μg ml^{-1} in the same diluent. An aliquot of internal standard solution (*p*-diethoxybenzene, 1.2 mg ml^{-1} in diluent) was added to the preparations to give an identical concentration of internal standard. Standard concentrations of 72 μg ml^{-1} each of I and internal standard were prepared in diluent. The chromatographic performance is controlled by maintaining the retention times of I and internal standard in windows of 7–11 and 13–16 min, respectively. A resolution factor ($R = 2\ (t_2 - t_1)/(W_2 + W_1)$, USP 23) of ≥ 8 was maintained by meeting these retention time windows. The retention time of each peak can be adjusted by increasing or decreasing the actonitrile content of the mobile phase; increasing the acetonitrile content decreases the retention time of both peaks. Replicate injections were made for the standard ($N = 5$, RSD for area ratios $\leq 2\%$) and the mean peak area ratio response was used. The strength of I in the sample preparation was calculated by the internal standard method.

Table 1
Intermediate precision for I in drug substance and drug product

	Drug substance (% I)	Drug product (% label claim)	
		25 mg capsules	200 mg capsules
N	10	10	10
Range	98.6–100.8	96.5–101.2	97.3–101.0
Mean	100.1	98.9	98.6
% RSD	±0.60	±1.9	±1.2

3. Results and discussion

The drug product of I is a formulation of the drug substance in capsules containing either 25 or 200 mg. I is determined in either the drug substance or in the drug product using the initial chromatographic eluent described under isocratic chromatographic conditions. The dose of I is controlled in the formulations by the capsule fill weight from a single capsule blend. Shown in Fig. 2 are typical chromatograms for the determination of I in the drug product. The stability indicating nature of the determination was demonstrated by stressing I under a variety of conditions. When the drug substance or drug product were stressed minimal degradation occurred and the mass balance was consistent with quantitative recovery of material. Degradates were well resolved from I and from the retention window of the internal standard. Shown in Fig. 3 are typical chromatograms for the drug product after it was stressed. These preparations were made without the addition of internal standard. The reliability of quantitating I was evaluated by determining linearity of the detector response, intermediate precision and recovery of I from the formulation placebo.

Table 2
Recovery of I from placebo

Addition level (%)[a]	% Recovery
49.7	101.7
50.4	102.5
101.0	101.0
101.3	102.6
153.3	101.9
150.0	101.0

[a] Percentage of the formulation level.

The detector response for I was linear ($n = 5$, correlation coefficient > 0.9999) from 24 to 118 μg ml^{-1} representing 33–164% of the assay level. The plot of peak area ratio (y) versus concentration I, μg ml^{-1} (x) gave a regression line with slope equal to 0.0136 and a y-intercept equal to 0.00054, which is not significant at the 95% confidence level.

Intermediate precision was evaluated for the determination of I. Precision data are presented in Table 1 for the drug substance and drug product. As shown, relative standard deviations for the determinations ranged from ±0.60 to ±1.9%. Accuracy of the determination was assessed by calculating the recovery of I after addition to the placebo at 50–150% of the formulation level. Results for the addition and recovery experiments performed in duplicate are shown in Table 2. Recoveries of 101.0–102.5% were observed.

Isocratic conditions provided acceptable determination of the intact drug. However, possible related substances in I which are either manufacturing impurities or degradates have a very large range of relative polarities. Gradient elution was necessary to retain the early eluting polar impurities while eluting the less polar impurities in a reasonable time. Robustness experiments showed that a low buffer concentration (0.01 M) provided adequate buffering capacity with no potential for salt precipitation when high concentrations of acetonitrile are used in the gradient profile. The ethyl ester of I, a nonpolar compound, is isolated as a precursor and is subsequently hydrolyzed and acidified to yield the drug substance as a hydrochloride salt. Other possible related substances which are more polar include a degradate formed by elimination of *N*,*N*-dibutylacetamide from I and mandelic acid which is used in the manufacture of I to resolve its enantiomers. Additionally,

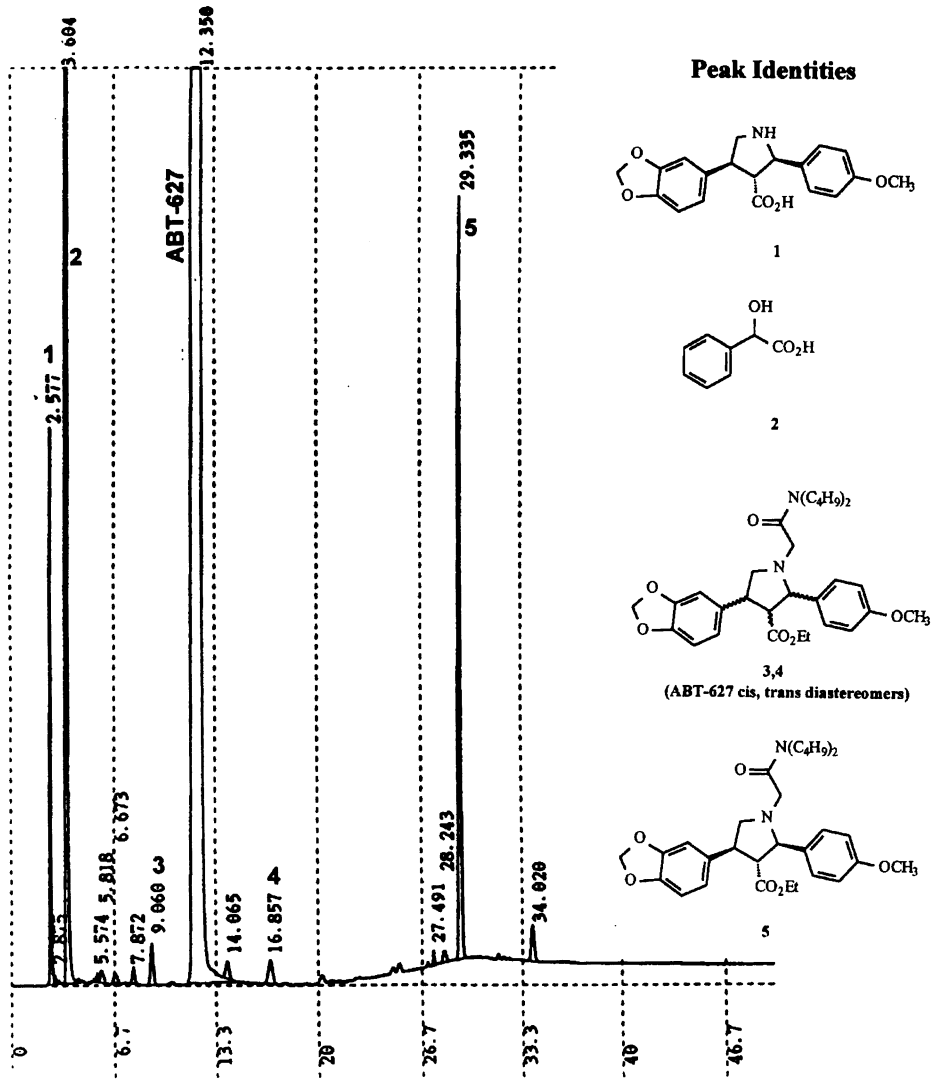

Fig. 4. Chromatogram of I spiked with known related substances. Peak identities: 1. *N*,*N*-dibutylacetamide elimination product; 2, mandelic acid; 3 and 4, diastereomers; 5, ethyl ester precursor.

the pyrrolidine ring of I has three asymmetric carbons, which can lead to four pairs of diastereomers. Chromatographic conditions were chosen to resolve these potential impurities in I as well as other unidentified impurities.

A chromatogram of I spiked with known related substances is shown in Fig. 4. As shown, the conditions provide a high degree of resolution with a run time of 50 min. Typical chromatograms for determining related substances in the drug substance and drug product are shown in Figs. 5 and 6, respectively. A retention window for I based on robustness data is specified to provide reproducible relative retention times (RRT's). The initial buffer/ acetonitrile ratio can be adjusted to meet this window. The buffer pH must also be controlled (2.9 ± 0.1) to maintain consistent RRT's for the late-eluting related substances that have vastly different polarities than I.

In order to assess the sensitivity of the method, solutions of the drug were prepared at 0.12 and 0.59 μg ml^{-1} (0.01 and 0.05% of the assay levels, respectively). These preparations were injected over an extended chromatographic run. At the 0.01% level, the drug is readily detectable (S/N approximately 15/1). However, at 0.01%, the drug is not acceptably quantitated due to the low peak response and the high variability of the integrated areas (RSD = ± 28%, $N = 7$). At 0.05%, quantitation is acceptable (RSD = ± 1.3%, $N = 6$). Therefore, the limits of detection and limit of quantitation are 0.01 and 0.05%, respectively.

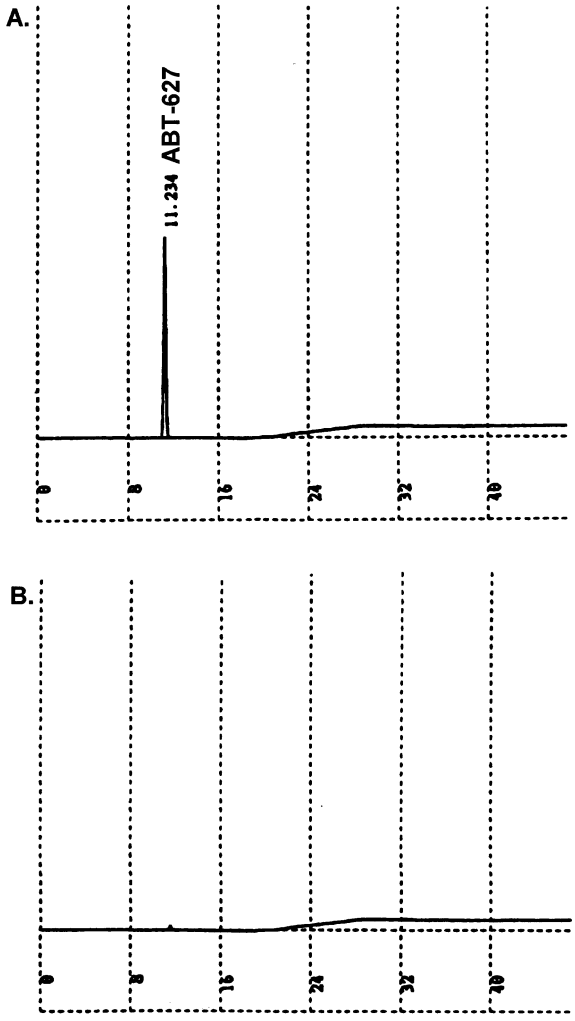

Fig. 5. Typical chromatogram for the determination of related substances in the drug substance and drug product. A, standard preparation (0.5% sample conc.); B, diluent blank.

Fig. 6. Typical chromatogram for the determination of related substances in the drug product. Peak identities are as shown in Fig. 4.

The reliability of the determination of related substances in I was evaluated by demonstrating linearity of the detector response and by determining the intermediate precision in the drug substance and drug product. The linearity for detector response of I was demonstrated for concentrations of 0.60–17.8 μg ml^{-1} (0.05–1.5% of the sample concentration). The plot of peak area (y) versus concentration I, μg ml^{-1} (x) was linear ($n = 5$, correlation coefficient > 0.9999), providing a regression line with a slope equal to 19805 and

Table 3
Intermediate precision for related substances[a]

RRT[b]	% Impurity	Mean	% RSD
A. Drug substance, Lot 1			
0.22(1)	0.24–0.25	0.25	±3.7
0.27	0.17–0.21	0.19	±8.8
0.34(2)	0.20–0.21	0.20	±2.1
0.40	0.16–0.20	0.19	±6.0
0.47	0.06–0.06	0.06	–
0.52	0.05–0.05	0.05	–
0.65	0.08–0.10	0.10	±8.9
0.75(3)	0.08–0.11	0.10	±12
0.83	0.09–0.12	0.11	±9.0
1.4(4)	0.11–0.15	0.13	±12
2.4	0.09–0.13	0.11	±12
B. Drug product (200 mg capsules) produced from Lot 1[b]			
0.22(1)	0.25–0.33	0.30	±11
0.25	0.14–0.19	0.30	±12
0.33(2)	0.18–0.22	0.19	±7.4
0.37	0.13–0.18	0.16	±12
0.45	0.05–0.07	0.06	±14
0.62	0.09–0.10	0.10	±5.5
0.75(3)	0.07–0.10	0.09	±13
0.90	0.06–0.09	0.08	±17
1.4(4)	0.12–0.15	0.14	±7.2
2.5	0.10–0.12	0.11	±8.2

[a] $N = 10$, by two analysts.
[b] Number in parentheses refer to compounds identified in Fig. 4, all other peaks are unknowns. All identified peaks are manufacturing impurities, except (1) which is a manufacturing impurity and a potential degradation product.

a y-intercept equal to 454, which is not significant at the 95% confidence level.

Intermediate precision was evaluated for the drug substance and drug product. The capsule formulation used in this study was prepared from the same lot of drug substance and these data are presented in Table 3. As shown, very comparable precision results were obtained for related substances in the drug substance and drug product. Authentic related substances for I have not been synthesized in sufficient quantities to perform standard addition and recovery at this time. However, the agreement for the quantitated amounts of related substances in Table 3 between the drug product and drug substance indicates quantitative recovery of the observed related impurities.

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