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Demonstrative validation study employing a packed column pressurized fluid chromatography method that provides assay, achiral impurities, chiral impurity, and IR identity testing for a drug substance

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Abstract

Assay development, assay validation, and documentation are reported here for a single packed column pressurized fluid chromatographic/ultraviolet (UV) method that provides: (1) simultaneous detection and quantification for the chiral drug, the chiral impurity and seven achiral impurities; and (2) a Fourier transform infrared (FT-IR) spectrometric identification test result for the Searle drug substance sample, xemilofiban. The separation is achieved in less than 30 min with three columns in tandem and a gradient of CO_2–CH_3OH. The post-column flow is split between UV (assay) and FT-IR (identification). Precision and accuracy are consistent within figures of merit obtained by liquid chromatographic-ultraviolet assays on analogous drug substances. The reported procedure combines three typical drug substance tests into one test (e.g. chiral impurities, achiral impurities, and infrared identification). © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Pressurized fluid chromatography (PFC)[1] is recognized as an appropriate method for the analysis of drug substances and products [1]. The impurity

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[1] The term pressurized fluid chromatography is preferred to supercritical fluid chromatography because the chromatographic conditions in so far as the mobile phase is concerned are not strictly supercritical.

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check of the drug substance is currently the most important analysis, thereby giving essentially the finger print of the chemical synthesis and of the stability studies. Today, the method of choice for assay and impurity analysis during development and production of drugs is reversed phase liquid chromatography (RP-HPLC) [2,3]. Anton and Siffrin [4] have emphasized, however, that orthogonal separation techniques are essential in the registration process to show that RP-HPLC gives reliable results. They have demonstrated that packed-column PFC with UV detection not only meets this requirement but is also a suitable tool for drug substance and drug product analysis. The re-examination of normal phase chromatography for high speed analysis has also been recently proposed [5]. The gradient separation conditions employed resulted in efficient separations in a fraction of the time necessary for comparable reversed phase separations.

The first application of PFC for separation of enantiomers was published in 1985 by Mourier et al. [6]. Since then, a number of PFC papers have been published which show superior efficiency and selectivity in a shorter time frame compared to HPLC. However, no publications seem to have appeared in the open literature regarding method validation. The absence of validated methods in the public domain fosters a situation where PFC is viewed rather cautiously for studying drug impurity profiles. While PFC methods are allowed to assume an aura of validity and authenticity, in practice they may never really become useful with samples that are typically encountered. Consequently, reports which describe assay validation studies employing real drugs using PFC should prove valuable and significant.

Assay validation ensures that an analytical methodology is accurate, specific, reproducible, and rugged throughout the specified range for which an analyte will be analyzed. The principles and procedures for validation of chromatographic methods are described in the U.S. Pharmacopeia [7]. Eight elements of method validation are listed: precision, accuracy, limit of detection, limit of quantification, specificity, linearity/range, ruggedness, and robustness. The Société Francaise Sciences et Techniques Pharmaceutiques has suggested a two step validation strategy: pre-validation and validation. An experimental design was described for each of the steps for the purpose of finding the most suitable way to determine the limits of detection, the calibration range and the optimum number of experiments to perform the validation phase [8]. The pressurize fluid chromatographic technique also has been suggested to fulfil the general requirements of current Good Manufacturing Processes (cGMP) environments and therefore be a suitable tool for drug substance analysis.

The objectives of this research were: first, to develop an achiral/chiral separation of xemilofiban and its impurities via PFC/UV; second, to provide an on-line PFC/FT-IR method for identification of xemilofiban; and third, to validate the assay for precision, accuracy, limit of detection, limit of quantification, specificity, linearity and range, robustness, and system suitability. Since this study is demonstrative in nature, exclusion of less prominent validation concepts, we feel, is not relevant to the value of the work. The validation of a test that combines three sample preparations (achiral impurities, chiral impurity, and infrared identification) is unique. The drug substance validation that we have performed may be used during drug development and before commercialization.

2. Experimental

2.1. Instrumentation

A Berger Instrument (Newark, DE) supercritical fluid chromatograph equipped with UV detector and high pressure flow cell was used for all separations. A mobile phase elimination interface (Lab Connections, Marlborough, MA) with Magna II FT-IR (Nicolet, Madison, WI) was used for identification of xemilofiban. A diol silica-based column (250 × 4.6 mm, 5 μm d_p) from Keystone Scientific (Bellfonte, PA) followed by two Chiralcel OD (250 × 4.6 mm, 10 μm d_p) columns from Chiral Technology (Exton, PA) were used in series for all separations. Air Products and Chemicals, Inc. (Allentown, PA) SFE/SFC grade CO_2 was used.

2.2. Sample preparation

Two different standard solutions were prepared for qualitative work. The first solution was prepared by dissolving 15 mg of xemilofiban (SC-54684A), 2 mg of its enantiomer (SC-55296A), and 2 mg of each impurity (SC-60604, SC-63485, SC-68223, SC-67235, SC-57174A, SC-64252, SC-54701B) in 10.0 ml of methanol. The second set of qualitative standards contained one of the individual compounds dissolved in 10 ml of methanol for a total of nine solutions. A third and fourth standard for quantification work at the assay level (e.g. 100 mg/10 ml CH_3OH) and impurity level (e.g. 0.5 mg/10 ml CH_3OH) was also prepared. Each solution before full dilution was sonicated for 20 min to obtain complete dissolution of the compound in methanol. After 20 min for sonication of the mixture, each sample was filtered using a 0.45 μm nylon Acrodisc (Gelman Science) disk. In order to prepare a solution for each batch of drug, 100 mg of drug was dissolved in 10 ml of methanol followed by sonication for 20 min. Next, each solution was analyzed and the concentration of impurities was determined. In order to determine the concentration of each impurity in various batches of drug, calibration curves for pure achiral xemilofiban with various concentration ranges were prepared. In other words impurities were quantitated versus the xemilofiban standards without correction for absorptivity differences.

The following chromatography conditions were used to obtain the desired separation of xemilofiban and the nine impurities:

Columns:	One diol and two chiralcel (250 × 4.6 mm OD) in series
CO_2 liquid flow:	3 ml/min
Outlet pressure:	120 atm
Temp.:	40°C
Modifier:	Methanol + 0.5% (v/v) isopropyl amine, IPA
Modifier program:	85/15%–45/55% (v/v) CO_2/MeOH at 2% min, hold for 5 min
Injection vol.:	75 μl for FT-IR detection and 3.2 μl for UV
Sample conc.:	10 μg/μl of xemilofiban and 0.2 μg/μl of each impurity
Detector:	UV, 275 nm

It was desirable to simultaneously have some means of peak identification; consequently, a mobile phase solvent elimination Fourier transform infrared interface was coupled to the column arrangement. For FT-IR detection, the column outlet flow was split into two portions. One portion of the flow was transferred to the UV detector while the other part was forwarded to FT-IR. The split ratio was set so that 20 parts of the flow passed through the UV and 1 part (approximately 70 ml/min of expanded CO_2 gas at 120 atm) was deposited on to the germanium disk of the FT-IR mobile phase elimination interface.

The specificity of our method was established by performing a blank injection at the beginning and end of a precision run. The injection procedure after injecting the blank was as follows: wash loop with 70 μl of the sample solution, wash loop a second time with approximately 60 μl of the same sample solution, fill injection loop with 3.2 μl of sample solution, inject 3.2 μl onto the column, wash loop twice with 200 μl methanol, and then re-inject the blank. A residual xemilofiban peak appeared in the second blank. Therefore, a blank injection has been performed in all our experiments if a different sample was to be subsequently examined.

3. Results and discussion

3.1. Preliminary studies

While published validation procedures are somewhat common for HPLC, analogous studies with packed column pressurized (supercritical) fluid chromatography are generally lacking. The potential for instrumentation capable of performing this type of separation in the pharmaceutical industry, however, has been recently demonstrated in our laboratory. The goal of our work

has been to determine how well pressurized fluid chromatography stands up in a validation study. The drug substance that was used is xemilofiban and its associated impurities. Fig. 1 illustrates the highly polar, chiral molecular structure of xemilofiban and several impurities. Not only does the parent material contain numerous heteroatoms but, in many cases the associated compounds are hydrochloride salts with a reactive acetylenic group.

Our initial concern was to perform a baseline chromatographic separation of the drug substance. Not only was separation of xemilofiban and all its impurities desired but the chiral enantiomer of xemilofiban was important in the separation process. A single stationary phase to accomplish isolation of all components was deemed not possible. Subsequently, three columns with two stationary phases were found to be satisfactory (e.g. one diol and two Chiracel OD). Unlike HPLC, coupling packed columns in PFC is quite feasible because column pressure drop across each column is relatively small and column flow is turbulent. The highly polar nature of the analytes coupled with the use of silica-based stationary phases dictated the use of methanol-modified CO_2 as the mobile phase. Isopropyl amine (IPA) was employed as an additive in order to both neutralize any amine salts that might be present and to attach to free silanol sites on the stationary phase thus helping to avoid peak tailing. To achieve our goal of baseline separation, a mobile phase methanol gradient (15–55%) was required. Since the temperature equalled only 40°C, the separation is more properly referred to as PFC rather than SFC. For development of the separation, an ultraviolet detector operating at 275 nm was employed.

Fig. 2 shows the separation of most of the components in the drug substance via packed column PFC/UV. Since all the impurity components were singly available, we could ascertain which compounds migrated down the packed column and were detected under the selected gradient conditions. SC-68224 and SC-55935 were either not eluted from the tri-column arrangement or were eluted but simply not detected at 275 nm. Nine components of the drug substance, however, eluted with near baseline resolution. Based upon single injections, peaks could be identified in the order of elution as: SC-60604, SC-63485, SC-68223, SC-55296A chiral enantiomer impurity, SC-54684A desired chiral xemilofiban, SC-67235, SC-57174A, SC-64252, and SC-54701B. It was interesting to observe that SC-68223 ($t_R = 13.26$ min) degraded with time. After 24 h at room temperature, the SC-68223 peak exhibited noticeably lower peak area and a new peak had appeared at 12.40 min. After 48 h under these conditions, the SC-68223 peak had completely disappeared.

In addition to the validation study, vide infra, that was performed using the above separation conditions, it was of interest to learn if using an auxiliary detector would provide similar information to what was learned employing UV detection. For this purpose, a less sensitive FT-IR detector was employed on-line. Albeit while sensitivity is lost, FT-IR does afford information concerning identification, and it responds to non-ultraviolet absorbing analytes. While a mass spectrometer would have probably been a better choice, this detector was unavailable. The tandem system eliminated the need for sample preparation for an infrared identity test and coupled chromatographic retention and spectroscopic identity testing.

Fig. 3 shows the Gram–Schmidt Reconstruction (e.g. FT-IR detection) of the standard mixture of xemilofiban and several impurities, first chromatographed, then flow-split between the UV and FT-IR detectors, and then deposited on a germanium disk for infrared assay. A similar number of peaks were detected with FT-IR as were detected with UV. Fig. 4 shows the co-added spectrum of xemilofiban eluted around 13.1 min which matched well with the infrared spectrum of an authentic sample of xemilofiban. A unique component eluted from the column which was FT-IR responsive with retention time of approximately 9 min but was not detected via UV. Fig. 5 shows the co-added spectrum of this unknown component which does not match well with the retention time of any of the known impurities; yet, it apparently is a strong infrared absorber. Split flow would not be used throughout the

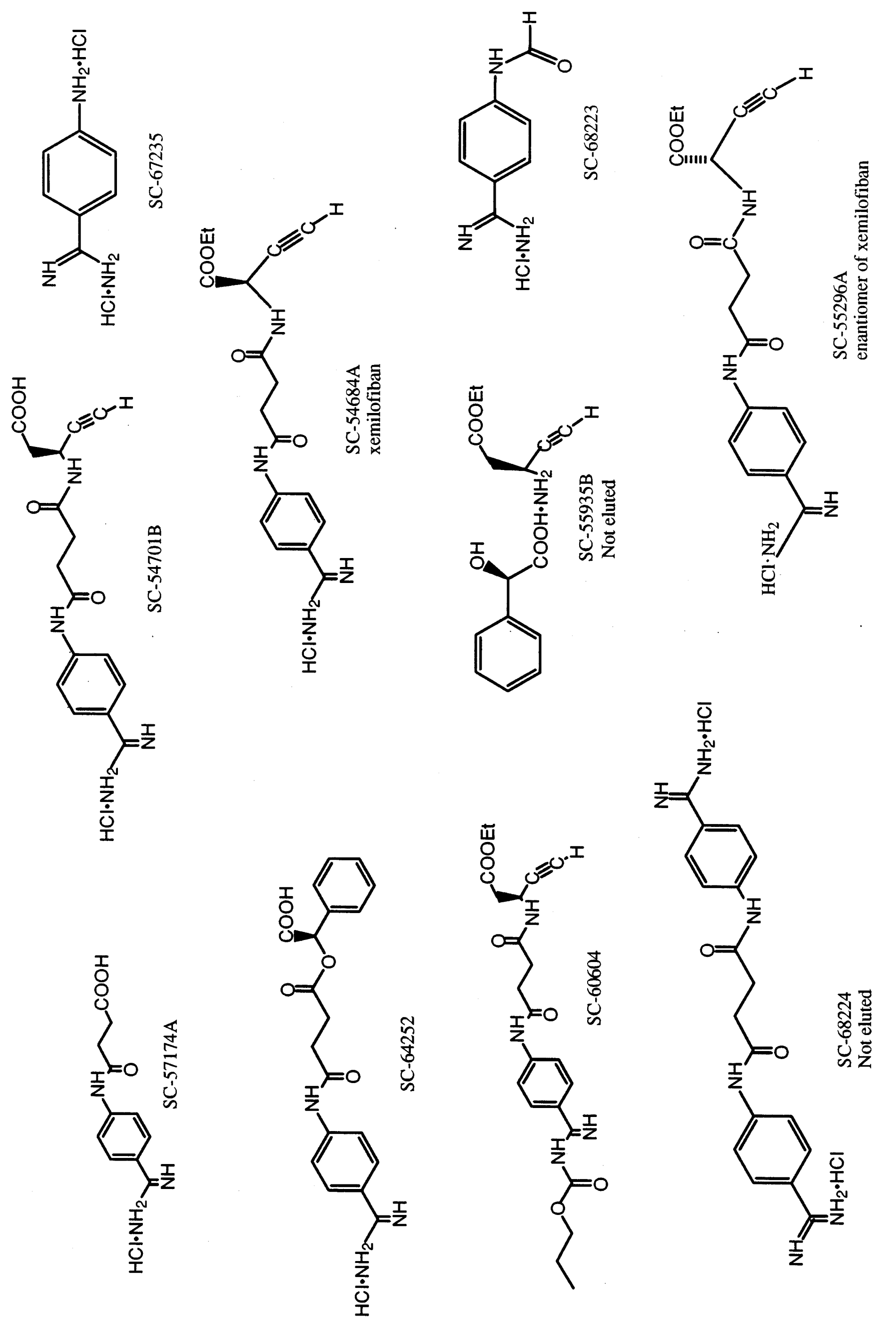


Fig. 1. Molecular structure of xemilofiban and impurities.

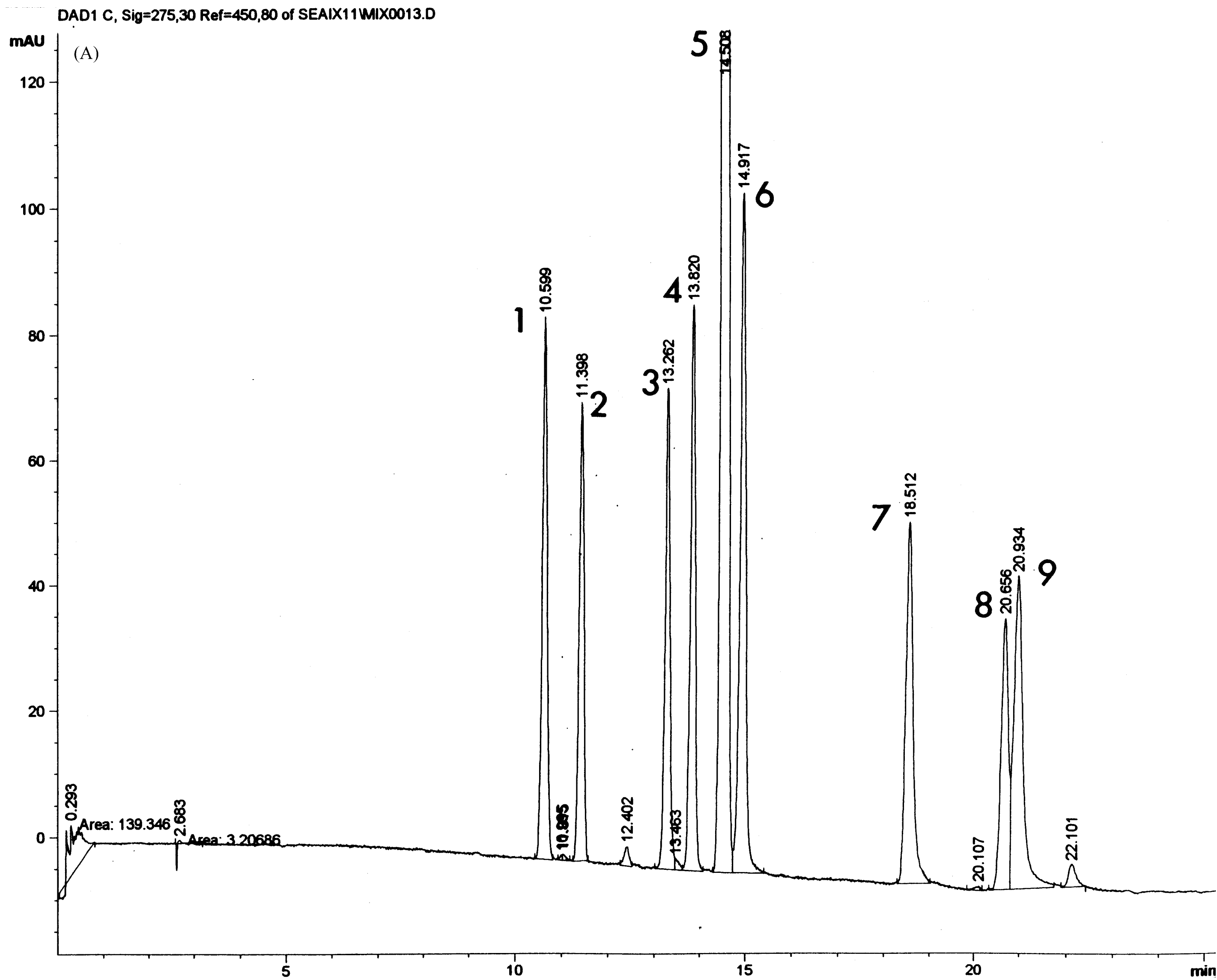


Fig. 2. Stability of xemilofiban and impurities. (A) SFC of freshly prepared xemilofiban and impurities. (B) SFC of xemilofiban and impurities after 24 h. 1, SC-60604; 2, SC-63485; 3, SC-68223; 4, enantiomer (SC-55296A); 5, xemilofiban (SC-54684A); 6, SC-67235; 7, SC-57174A; 8, SC-64252; 9, SC-54701B.

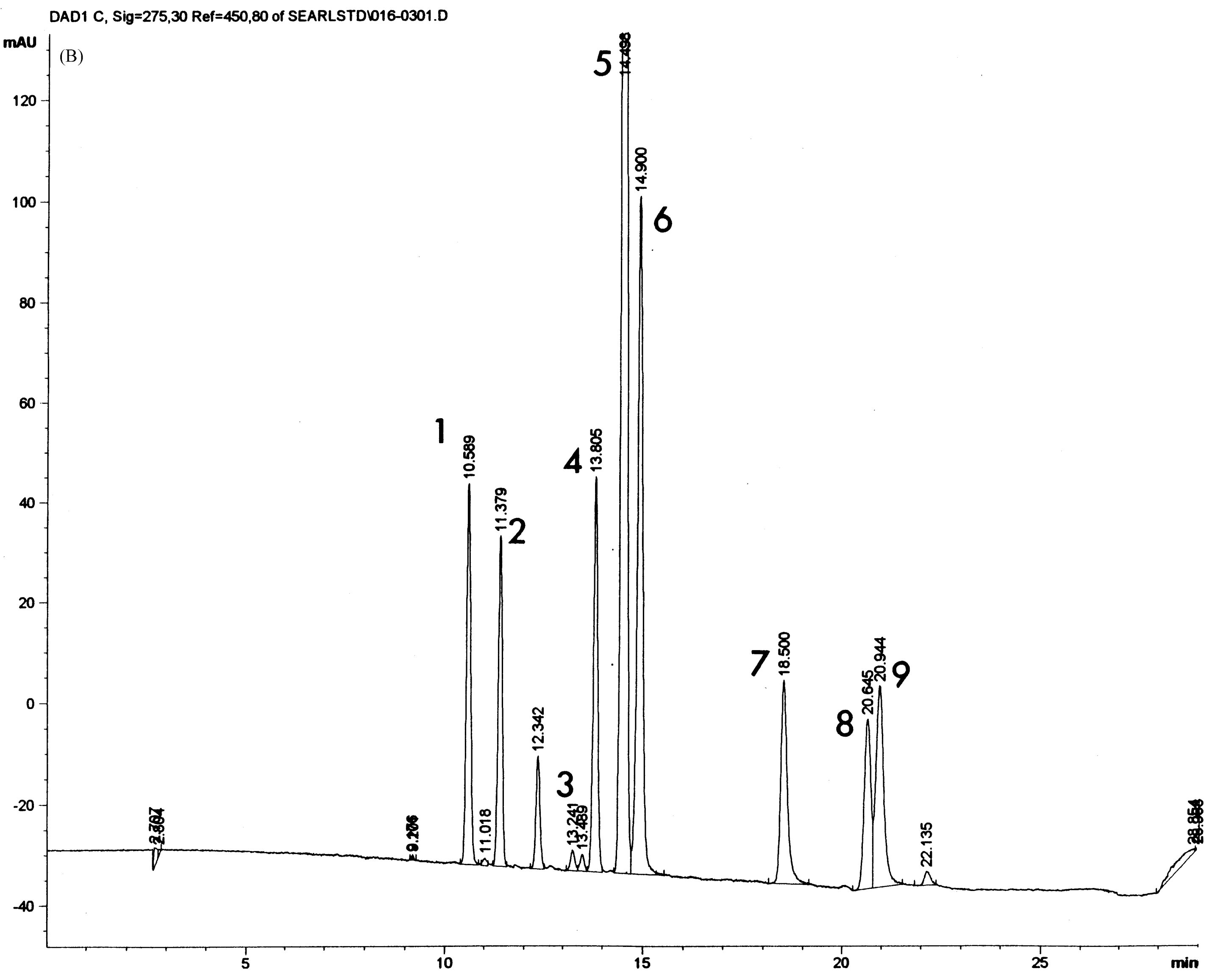


Fig. 2. (*Continued*)

assay. Split flow would typically be used during 'one' of the chromatographic runs during analysis to generate IR spectra of the major component and confirm the identity.

To summarize our findings to this point, we have reported a single pressurized fluid chromatographic method using three tandem packed columns which can be completed in less than 30

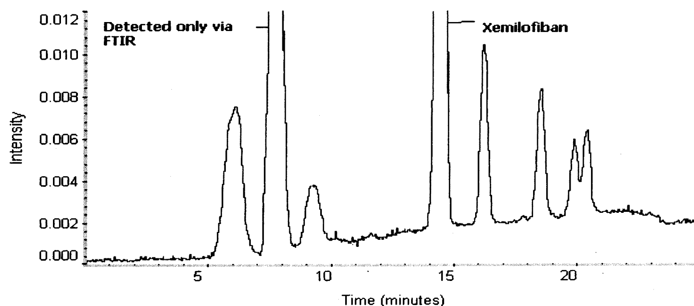


Fig. 3. On-line Gram–Schmidt SFC/FT-IR of xemilofiban and impurities.

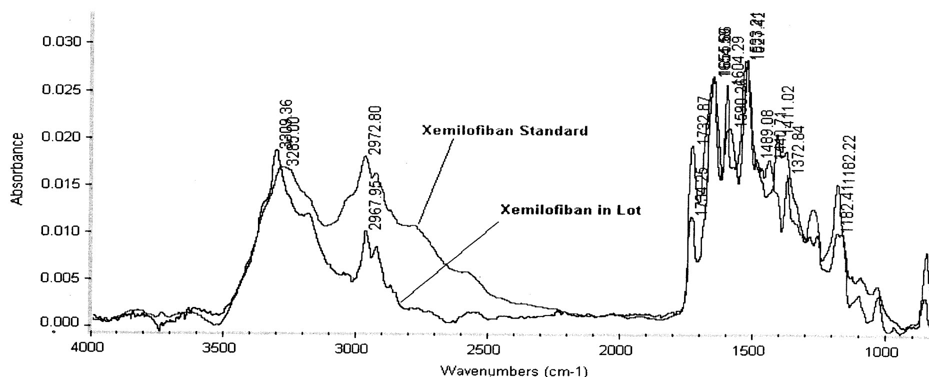


Fig. 4. FT-IR spectra of standard xemilofiban and xemilofiban in a lot.

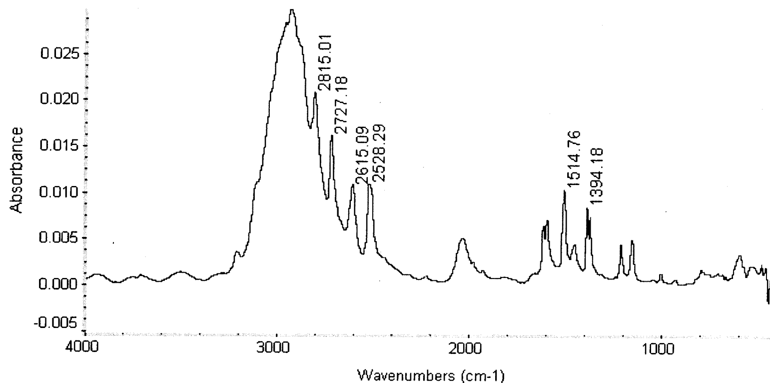


Fig. 5. FT-IR spectrum of an unknown peak eluting at $T = 9$ min.

Table 1
Selectivity tests with prepared mixtures

	Day 1 mixture			Day 2 mixture		
	Avg. raw area	%RSD	Conc. (mg/10 ml)	Avg. raw area	%RSD	Conc. (mg/10 ml)
SC-60604	188	0.53	0.64	198	1.16	0.67
SC-63485	165	2.12	0.56	174	1.99	0.58
SC-68223	266	0.37	0.91	242	1.71	0.82
Chiral impurity[a]	156	4.35	0.53	160	0.95	0.54
Xemilo[b] (Chiral)	28 375	1.03	96.94	29 085	1.82	97.74
SC-67235	190	0.30	0.65	245	6.63	0.83
SC-57174	185	3.74	0.63	197	4.15	0.66
SC-64252 + SC-54701	270	2.42	0.92	288	3.60	0.97

[a] Each impurity spiked at 0.5% assay level (i.e. 10 μg/μl × 0.005 = 0.05 μg/μl).
[b] Response factor assumed to be same for all species.

min, and which yields: (a) separation of many known drug impurities; (b) measurement of chiral impurity concentration; and (c) identification of major components in the drug substance. Our goal in the second half of this manuscript will be to demonstrate that the method can be satisfactorily validated in terms of precision, accuracy, limit of detection, limit of quantification, specificity, linearity and range, robustness, and system suitability.

3.2. Selectivity

Selectivity is defined as the ability to accurately and specifically measure the analyte of interest in the presence of other components that may be expected to be present in the sample matrix. For this purpose, a standard mixture of xemilofiban (100 mg/10 ml) was spiked with all known soluble impurities (total = 8) in the drug substance at the 0.5% level. On two different days, an individual solution was prepared. Table 1 shows the calculated concentration, percent relative standard deviation (RSD), and raw area of xemilofiban and each impurity measured on each of the 2 days. It should be noted that the response factor for xemilofiban was used to calculate each impurity concentration. The combined concentration of SC-54701 and SC-64252 is reported since our method of separation did not completely resolve these two components. Concentration is expressed in mg/10 ml of solution. Ideally, each impurity should have been noted at 0.5 mg/10 ml, however, the concentration of each measured impurity ranged from 0.53 to 0.83 mg/10 ml or approximately 0.5–0.8% of the xemilofiban concentration. For triplicate injections, RSDs were below 5% with one exception.

3.3. Linearity and range

Linearity is defined as the ability of the method to elicit test results that are directly proportional to analyte concentration within a given range. Range is the interval between the upper and lower concentration levels of analyte to be determined in the method. For the determination of xemilofiban linearity, eight levels of standard were employed ranging from 20 to 300% of the assay concentration (10 μg/μl or 32 μg/3.2 μl injection). For example, at the 20% level, 6.4 μg (10 μg/μl × 3.2 μl × 0.20) of xemilofiban was injected. Employing the initial six points (Fig. 6A) yielded $R^2 = 0.995$, intercept = + 1388 area, slope = 0.809 area/ng). Fig. 6B shows the calibration plot for chiral xemilofiban near the impurity level concentrations. These concentrations here ranged from 0.025 (8 ng injected) to 2.0% (640 ng injected) of the assay concentration. The linearity parameters were $R^2 = 0.9998$, intercept = + 4.03 area, slope = + 0.899 area/ng. The response factor for xemilofiban appears to be slightly higher at impurity levels than at assay level as judged by the calculated slope of the linear regression. The

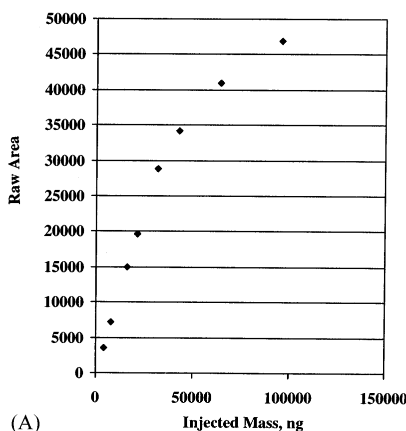
linearity of the chiral impurity response was also determined at five levels (0.025–5.0%) of the assay concentration. Over this range $R^2 = 0.9999$, intercept = + 5.30 area, slope = + 0.933 area/ng.

3.4. Limit of detection and quantification

The limit of detection (LOD) is defined as the concentration that produces a peak height of three times the signal-to-noise ratio (S/N). However, limit of quantification (LOQ) is defined as the minimum concentration of analyte that can be determined with acceptable precision and accuracy. Usually a signal-to-noise ratio of 10-to-1 is used to determine LOQ. Consequently, the data gathered from our linearity study suggest that the limit of detection for chiral xemilofiban is 0.025% of assay or 8 ng injected on column. Not surprisingly, the chiral impurity SC-55296A yielded a similar LOD.

3.5. Accuracy and precision

Accuracy is measured based on exactness of an analytical method or the closeness of agreement between the measured value and the value accepted either as a true or conventional value. Precision is measured based on the degree of repeatability of an analytical method under normal operation and normally is expressed as percent RSD. It is usually determined from a minimum of nine determinations commonly with three samples at three concentrations with three repetitions per each.

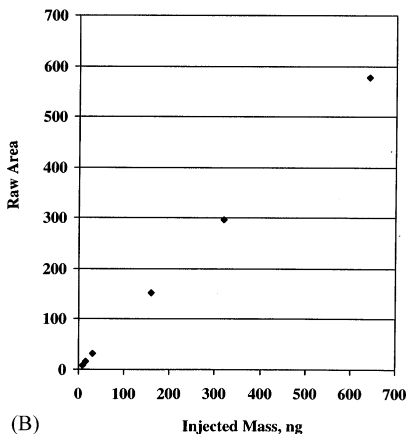


- **Five levels of Xemilofiban standard from 20% to 120% of assay concentration which was 10mg/mL or 32µg/ µL injection**

R^2 = 0.9997 (five points)

Slope = 897.6

Intercept = +275.7



- **Five levels of Xemilofiban standard from 2% to 0.025% of assay concentration**

R^2 = 0.9998 (six points)

Slope = +2.87

Intercept = +4.03

LOD = 8 ng

Fig. 6. (A) Parent linearity at assay level. (B) Parent linearity at impurity levels.

Table 2
Assay and linearity precision/accuracy for authentic sample prepared at 80, 100, and 120% of the assay concentration (10 μg/μl)[a]

Conc.	Lot 93K019-H2B Day 1 (mg/10 ml)										
	60604	63485	68223	Chiral imp.	Xemi	67235	57174	64252	54701	others	Total
80%											
Sample 1	0.25	0.13	0.05	0.28	77.98	0.00	0.37	0.00	0.13	0.42	79.62
Sample 2	0.24	0.14	0.05	0.27	77.21	0.00	0.36	0.00	0.13	0.38	78.78
Sample 3	0.26	0.14	0.05	0.29	80.04	0.00	0.40	0.00	0.14	0.41	81.72
Ave.	0.25	0.14	0.05	0.28	78.41	0.00	0.38	0.00	0.14	0.40	80.04
%RSD	2.37	1.88	5.39	3.62	1.87	–	5.00	–	4.37	5.34	–
100%											
Sample 1	0.31	0.16	0.06	0.35	98.43	0.00	0.48	0.00	0.15	0.52	100.47
Sample 2	0.31	0.20	0.06	0.36	98.20	0.00	0.51	0.00	0.15	0.51	100.28
Sample 3	0.32	0.18	0.06	0.36	98.90	0.00	0.48	0.00	0.15	0.50	100.96
Ave.	0.31	0.18	0.06	0.36	98.51	0.00	0.49	0.00	0.15	0.51	100.57
%RSD	2.18	9.76	3.89	0.98	0.37	–	3.38	–	2.84	2.21	–
120%											
Sample 1	0.39	0.23	0.07	0.44	114.48	0.00	0.60	0.00	0.19	0.57	116.97
Sample 2	0.39	0.24	0.07	0.45	114.66	0.00	0.60	0.00	0.18	0.57	117.15
Sample 3	0.39	0.21	0.06	0.44	114.39	0.00	0.57	0.00	0.18	0.53	116.79
Ave.	0.39	0.23	0.07	0.44	114.51	0.00	0.59	0.00	0.18	0.56	116.97
%RSD	0.86	5.62	7.50	1.17	0.12	–	2.56	–	2.79	4.18	–

[a] Raw area standardization: average peak area counts = 29 418 (five injections) RSD = 1.18%.

Authentic sample lots of xemilofiban were available to us for conducting precision/accuracy studies. Lot 93K019-H2B was selected. On day # 1, three individual and unique samples were weighed out and dissolved at three concentration levels (e.g. 80–100–120% of assay concentration). Table 2 shows the measured concentration of xemilofiban, the chiral impurity, and seven known impurities. Average impurity level and RSD are also listed for triplicate injections at each of the three concentrations. Impurities SC-67235 and SC-64252 were not detected. A number of unidentified peaks were observed. Their combined peak areas were summed and a collective concentration was calculated and listed as ‘others’. If the lot sample had been 100% pure chiral xemilofiban, the measured concentrations would have been 8, 10, and 12 μg/μl. Obviously, our measured values do not match these numbers. What might be more important is the high precision that was achieved among the three samples taken at each xemilofiban concentration (e.g. 1.87, 0.37, 0.12%). RSDs for the impurities are higher but, in general, are within acceptable limits. Since the measured impurities are believed to originate from xemilofiban, it was of interest to determine the total concentration of all components in the lot. For the 80 and 100% concentrations, the totals (80.04 mg/10 ml and 100.57 mg/10 ml) agreed exceptionally well with expected values.

Day # 2 results with three samples per concentration gave analogous results. Xemilofiban values at 100% assay concentration were 98.51 mg/10 ml (day # 1) RSD = 1.87% and 98.73 mg/10 ml (day # 2) RSD = 1.48%. Summed total concentration of all components was 100.57 mg/10 ml (day # 1) and 100.75 mg/10 ml (day # 2).

It was of interest to perform 1-day linearity studies for the SC-67235 and SC-60604 impurities spiked into authentic xemilofiban lot samples at 0.025–2.0% levels. Lot 93K019-H2B was once again used. In the case of SC-60604, approximately 90 ng was found in the original lot, while no SC-67235 was observed in the lot. The spiked mass ranged from 8 to 640 ng. The calibration plots created from the measured peak areas for

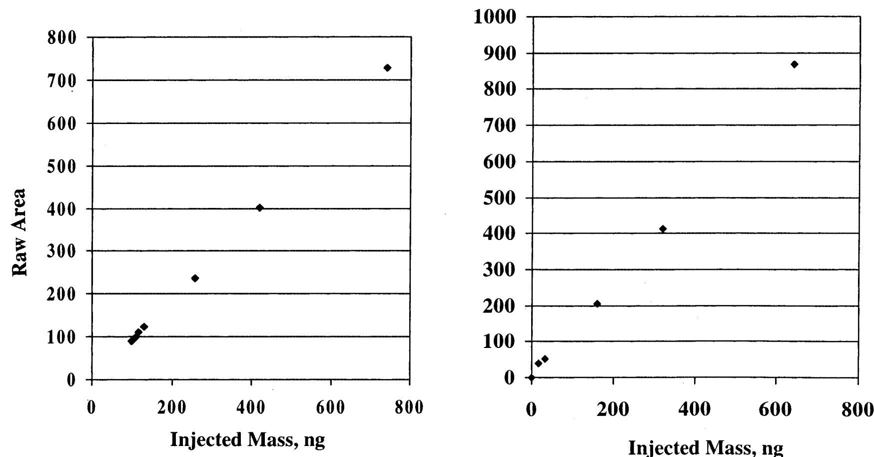


Fig. 7. Linearity of SC-60604 (left) and SC-67234 (right) spiked into authentic xemilofiban sample — day 1. SC-60604: slope = 0.993 area/ng, intercept = −9.520 area, $R^2 = 0.9994$; SC-67235: slope = 1.343 area/ng, intercept = −0.241 area, $R^2 = 0.9984$.

each impurity are shown in Fig. 7. Intercepts and slopes for the two impurities are −9.5201 area and +0.9929 area/ng (SC-60604) and −0.2407 area and +1.3433 area/ng (SC-67235). In order to complement the linearity study, a 2-day precision study was conducted with SC-60604 spiked into the same sample lot. Table 3 lists the results obtained on day #1. Three different samples from the same lot were spiked at 0.5% assay concentration. PFC/UV was carried out, and the SC-60604 peak area was determined after background correction. Concentrations of 0.506, 0.498, and 0.503 mg/10 ml were measured, which compared quite well with the expected value of 0.500 mg/10 ml, since the response factor for SC-60604 was assumed to equal that of xemilofiban. Day #2 of the precision study on three new samples gave similar values for SC-60604 (e.g. 0.50, 0.50, and 0.49 mg/10 ml) with an RSD less than one.

The PFC/UV assay method that was developed has been applied to two different lots of xemilofiban. In order to obtain some sense of repeatability, three samples of each lot were examined. Xemilofiban assay values for lot 94K035-H2A were 97.82, 96.79, and 99.12 mg/10 ml; while, the values for lot 94K021-H1A were considerably tighter (e.g. 99.26, 98.57, and 98.98 mg/10 ml), Table 4. Good precision was obtained for the chiral impurity in both lots although the impurity in 94K035-H2A was slightly higher than that found in lot 94K021. For the other impuri-

Table 3
Two day precision study for SC-60604/spiked xemilofiban samples day 1[a]

	Avg. area SC-60604	Avg. area xemilofiban	SC-60604 area after background correction	Conc. mg/10 ml
Lot with no SC-60604 added	100	30 554	0	
Lot with 0.5% SC-60604 added, #1	261	30 564	161	0.51
Lot with 0.5% SC-60604 added, #2	258	30 548	158	0.50
Lot with 0.5% SC-60604 added, #3	260	30 739	160	0.50
			Ave.	0.50
			%RSD	0.79

[a] Day 2 data was similar.

Table 4
Assay and impurity levels for three different samples of two different xemilofiban lots[a]

Analyte/lots	Lot 94K035-H2A (sample # 1)	Lot 94K035-H2A (sample # 2)	Lot 94K035-H2A (sample # 3)	Lot 94K021-H1A (sample # 1)	Lot 94K021-H1A (sample # 2)	Lot 94K021- H1A (sample # 3)
SC-60604	0.03	0.03	0.03	ND	ND	ND
SC-63485	ND	<0.03	<0.03	0.08	0.07	0.08
SC-68223	0.05	0.05	0.05	ND	ND	ND
Chiral imp.	0.27	0.27	0.28	0.20	0.21	0.19
Xemilofiban	97.82	96.79	99.12	99.26	98.57	98.98
SC-67235	0.07	0.08	0.06	ND	ND	ND
SC-57174	0.34	0.33	0.35	0.21	0.21	0.22
SC-64252	ND	ND	ND	ND	ND	ND
SC-54701	0.14	0.13	0.13	0.12	0.14	0.12
Others	0.14	0.15	0.14	0.14	0.16	0.17
Total	98.86	97.84	100.18	100	99.36	99.76

[a] ND, not detected. Units are mg of analyte/10 ml of solution.

ties, the assay values were similar without regard to the lot number. An excellent mass balance was achieved with lot 94K021-H1A (e.g. 99.70%). For lot 94K035-H2A, the average mass balance was 98.92%. The number of other identifiable impurities which could be assayed varied between five and seven. In these cases, the consistency of analysis was very strong between the two lots. Clearly, the reported assay method is applicable to xemilofiban drug samples.

In conclusion, PFC has proven to be a viable separation technique for validation of a drug substance. In addition to affording baseline separation of most all of the drug impurities, the methodology also yielded a measure of the chiral purity with a single injection. The low pressure drop typically across a packed column with a mobile phase of predominantly CO_2 afforded us the opportunity to stack columns of varying stationary phase. Since the mobile phase spontaneously vaporizes, mobile phase elimination FT-IR could be readily performed post-column to provide identification of various analytes as well as to detect non-UV-absorbing analytes. PFC, therefore, appears to complement reversed phase chromatography quite well for pharmaceutical analysis. The ability to interface a variety of detectors to packed column PFC is a distinct advantage.

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