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Characterization and resolution of reversed phase HPLC chromatography failure attributed to sulfobutylether-β-cyclodextrin in a pharmaceutical sample preparation

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ABSTRACT

A reversed phase HPLC method developed for a drug product formulation using hydroxypropyl-β-cyclodextrin (HPCD) was rendered ineffective for analyzing a similar formulation containing sulfobutylether-β-cyclodextrin (SBECD). The active pharmaceutical ingredient (API) and the majority of its impurities became more strongly retained, eluting as an incoherent conglomerate of peaks. Furthermore, this phenomenon was reproduced in subsequent injections of the API reference standard. Based on HPLC and LC–ESI-MS studies, the chromatography failure was attributed to the accumulation of SBECD on the HPLC column. The subsequent interaction of the API with bound SBECD resulted in the aberrant chromatography. An anion-exchange solid-phase extraction treatment was developed and qualified to selectively remove SBECD from sample solutions, thereby allowing the same HPLC method to be used. The sample treatment procedure exhibited suitable accuracy and precision for quantitating the API and its impurities, and resulted in typical chromatographic profiles.

1. Introduction

Cyclodextrins are cyclic oligosaccharides with a conformation resembling a conical cylinder or toroid [1,2]. Primary and secondary hydroxyls are positioned at either edge of the toroid, respectively, resulting in an interior that is less hydrophilic than the surrounding aqueous environment [1,2]. These unique characteristics of cyclodextrins enable host–guest interactions with hydrophobic molecules, resulting in inclusion complexes that change the solubility of the guest molecules. Industrially produced cyclodextrins include α, β and γ types (comprised of six, seven and eight glucose subunits, respectively), which have also been substituted at the hydroxyl proton or hydroxyl group to alter their physicochemical properties [1]. Cyclodextrins have been evaluated as pharmaceutical excipients to improve the solubility, stability, bioavailability and aesthetic qualities of drug product formulations [2–6]. Furthermore, cyclodextrins are employed as chiral selectors for separations by HPLC, GC, CE, TLC and other techniques [7–10].

Sulfobutylether-β-cyclodextrin (SBECD) and hydroxypropyl-β-cyclodextrin (HPCD) consist of varying degrees of hydroxyl proton substitution with $CH_2(CH_2)_3OSO_3^-$ and $CH_2CH(OH)CH_3$ functional groups, respectively. Previously, HPCD was shown to increase the aqueous solubility and photostability of tretoin (all-*trans*-retinoic acid) [11], whereas SBECD increased the bioavailability of the steroid danazol from buccal tablets [12]. These cyclodextrins were evaluated in formulation development for a drug substance (MW > 1500 Da), consisting of a glycopeptide (vancomycin) core modified with hydrophobic (R_1) and hydrophilic (R_2) sidechains (Fig. 1).

An effective reversed phase (C18) gradient HPLC/UV method employing acidified (0.05% formic acid) acetonitrile–water mobile phases was previously developed using a design of experiment approach and was validated to support the commercial manufacturing of both the drug substance and a drug product containing HPCD. This separation adequately resolved 30+ impurities from the active pharmaceutical ingredient (API) peak and was proven to be rugged by its successful implementation in several affiliated laboratories. Therefore, retaining the existing HPLC method was of utmost interest in order to evaluate the new SBECD and HPCD formulations. The presumption was this method would be equally effective for these formulations. The ensuing observation of aberrant chromatography in the SBECD-formulated samples was unexpected and inspired efforts to better understand its cause.

This article presents: (1) the characterization of the chromatography failure by HPLC/UV and HPLC with electrospray MS (HPLC–ESI-MS) and (2) the development and qualification of an effective anion-exchange solid-phase extraction (SPE) procedure to selectively remove SBECD prior to HPLC analysis. Because of the common utilization of acidified acetonitrile–water mobile phases

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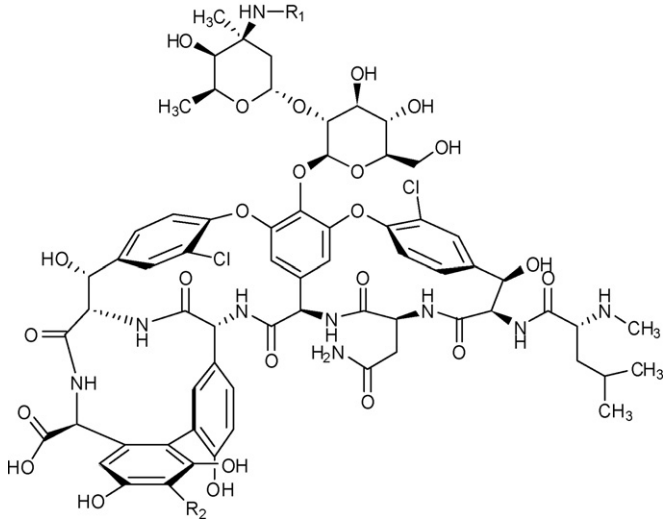

Fig. 1. Partial structure of developmental glycopeptide drug substance, consisting of a vancomycin core modified with hydrophobic (R_1) and hydrophilic (R_2) sidechains.

in reversed phase HPLC and increased use of SBECD in drug product formulations, it is of interest to understand the nature of the chromatography failure and to demonstrate effective, alternative analytical methodologies.

2. Experimental

2.1. Materials

The excipients SBECD (sulfobutylether-β-cyclodextrin), HPCD (hydroxypropyl-β-cyclodextrin) and mannitol were purchased from Cydex (Overland Park, KS), Roquette (Keokuk, IA) and MultiPharm/EM Science (Gibbstown, NJ), respectively. All solvents were HPLC grade or better and purchased from Burdick & Jackson (Muskegon, MI). Formic acid (ACS reagent grade) was purchased from EMD (Gibbstown, NJ). All reagents were used without further purification.

2.2. Solid-phase extraction

Drug product solutions containing 0.5 mg/mL of API, 1.6 mg/mL of SBECD and 0.6 mg/mL of mannitol were prepared in acidified (0.05% formic acid) acetonitrile–water (10:90, v/v) diluent. Aliquots (4 mL) were drawn through J.T. Baker BakerBond SAX (500 mg, 3 mL) quaternary amine SPE cartridges (Phillipsburg, NJ) at a rate of approximately 1 mL/min using a vacuum manifold; cartridges were rinsed with an additional 5 mL of diluent. The combined sample and rinsate (~9 mL) were collected in 10 mL volumetric flasks and diluted to volume to yield a 0.2 mg/mL nominal API solution for HPLC analysis.

Samples were quantitated by HPLC/UV against an API reference standard solution (0.2 mg/mL in acetonitrile–water–formic acid, 5:95:0.05, v/v/v) that was prepared from the same drug substance material. The HPLC/UV method parameters are summarized in Table 1.

2.3. Characterization of SBECD-induced chromatography failure

Since SBECD and HPCD lack UV chromophores, mass spectrometry was utilized to determine the HPLC elution profile of each. Injections of HPCD only or SBECD only samples (2 mg/mL in acetonitrile–water–formic acid, 2:98:0.05, v/v/v) were bracketed within injections of diluent blank and API reference standard for HPLC analysis. Samples were analyzed with a Thermo Finnigan (San Jose, CA) LXQ ion trap mass spectrometer, which was connected in series with an Agilent 1100 HPLC system and photodiodearray detector (Agilent, Wilmington, DE); refer to Table 1 for the HPLC/UV instrument parameters. The mass spectrometer was equipped with an electrospray ionization source operating in the negative-ion mode (ESI^-) for both SBECD and HPCD analyses. The sheath gas (nitrogen) flow was set at 30 (arbitrary unit), and the auxiliary gas flow at 5. The capillary temperature was set at 275 °C. The capillary voltage was maintained at 5 kV. Furthermore, the HPLC column performance was verified by inspecting the HPLC/UV chromatograms of API reference standard injections bracketing the HPCD and SBECD runs.

Table 1
Summary of HPLC/UV method and instrument parameters.

Column	Waters SunFire 3.5 μm C18, 150 mm × 4.6 mm
Autosampler temperature	5 °C
Column temperature	30 °C
Wavelength	230 nm
Mobile phase A	2:98:0.05 (v/v/v) (acetonitrile:water:formic acid)
Mobile phase B	60:40:0.05 (v/v/v) (acetonitrile:water:formic acid
Injection volume	80 μL
Flow rate	1.0 mL/min

Gradient

Time (min)	Mobile phase A	Mobile phase B
0	90	10
20	85	15
30	80	20
50	60	40
50.5	0	100
55.5	0	100
56	90	10
65	90	10

To evaluate whether the chromatography failure was specific to the Waters SunFire C18 column, three additional reversed phase HPLC columns with similar dimensions (150 mm length × 4.6 mm i.d.) and different particle substrate (i.e., polymeric, silica or hybrid) and ligand types (i.e., C18 or embedded polar RP18) were evaluated. The properties of each column are summarized and compared in Table 2. After each column was equilibrated, a sequence of HPCD only and then SBECD only samples (each 2 mg/mL in acetonitrile–water–formic acid, 2:98:0.05, v/v/v) bracketed between API reference standard injections, was run to evaluate column performance.

The specificity of the chromatography failure to the API itself was evaluated by analyzing a structurally dissimilar molecule (Compound X). The HPLC method was identical to that used for the API, except for removing the 50 min step of the gradient (Table 1) to elute Compound X ahead of the wash step of the gradient and performing 20 μL sample injections. The Compound X sample (0.2 mg/mL in acetonitrile–water–formic acid, 2:98:0.05, v/v/v) was analyzed on two different SunFire C18 columns, including one with no prior SBECD treatment and a second in which SBECD samples were previously run. The API reference standard solution was also run on each column for comparison.

3. Results and discussion

3.1. Observation of aberrant chromatography and column regeneration

Typical chromatograms of the API reference standard and HPCD-formulated sample are shown in Fig. 2B and C, respectively. The API peak elutes at ~23 min and numerous low level impurity peaks are observed. A blank chromatogram is included for comparison in

Table 2
Comparison of evaluated reversed phase, 150 mm length × 4.6 mm i.d. HPLC columns (adapted from product literature or certificate of analysis).

Column	Substrate	Ligand	Particle size (μm)	Carbon load (%)	Pore size (Å)	Surface area (m^2/g)
SunFire C18 (Waters, Milford, MA)	Silica	C18	3.5	16.63	86	368
PLRP-S (Polymer Laboratories, Amherst, MA)	Polystyrene/divinylbenzene	None	3	Not applicable	100	414
SymmetryShield RP18 (Waters, Milford, MA)	Silica	C18 with embedded carbamate	3.5	17.64	95	344
Xterra C18 (Waters, Milford, MA)	Silica/polymer hybrid	C18	3.5	15.31	130	183

Fig. 2A. However, the SBECD-formulated sample produced a broad, poorly defined peak (apex ~29 min), as well as the absence of nearly all impurities (Fig. 2D). Repeat SBECD sample injections resulted in increasing retention time for the main peak, ultimately appearing as a split peak eluting partially in the gradient wash step (see Fig. 2E). The aberrant chromatography was reproducible even in subsequent injections of API reference standard only. Furthermore, API reference standard injections (0.1 mg/mL) spiked with just 3 μg/mL of SBECD (equivalent to 0.25 μg or 0.5% of nominal level in an 80 μL injection) produced a visually distinguishable broadening of the API peak within ten injections; this corresponded to both a gradual decline in peak height (4.2×10^5 to 3.9×10^5 μAU) and a gradual increase in tailing factor (2.9–3.4).

The specificity of the chromatography failure towards the SunFire C18 column was evaluated using different reversed phase sorbents of varying substrates and ligand types. Stacked chromatograms of the API reference standard injected prior to HPCD (Fig. 3A), after HPCD (Fig. 3B) and after SBECD (Fig. 3C) are presented for each of the HPLC columns. The chromatographic profiles were unchanged upon injecting HPCD into each column, but aberrant chromatography was observed in varying degree in all four columns each after injecting SBECD. The effect of SBECD on the embedded carbamate SymmetryShield RP18 column was comparable to that of the SunFire C18 column, including the absence of a coherent API peak. By contrast, the polystyrene/divinylphenyl polymeric PLRP-S and the polymer-silica hybrid Xterra C18 columns each exhibited an API peak shift to higher retention time along with more pronounced broadening and tailing. These changes in chromatography are also quantitatively characterized as capacity factor, tailing factor, peak height and peak width parameters for each run in Table 3. Therefore, the chromatography failure is not unique to the SunFire C18 sorbent.

No similar SBECD-related disruptions to HPLC reversed phase separations have been documented in the literature. Furthermore, no such chromatographic effects for reversed phase separations employing acidified acetonitrile–water mobile phases were disclosed in personal communications with Cydex, Inc., which is the exclusive manufacturer of SBECD.

A column regeneration procedure consisting of an isocratic flow (1.0 mL/min) of methanol–0.1 M phosphate, pH ~ 4.5 (10:90, v/v) for 2 h was successfully implemented for several compromised SunFire C18 columns. The restoration of column performance was verified by obtaining a typical impurity and API peak profile from API reference standard injections. Irreversible damage may eventually result for columns that have been repeatedly compromised by SBECD. Raising the pH above the pK_a of silanols (pK_a ~ 3.5) could disrupt the retention of SBECD, possibly by anionic charge repulsion from the SBECD sulfonate groups. This is consistent with a previous analysis of SBECD by reversed phase HPLC using only unbuffered water as mobile phase [13], resulting in extremely poor retention and elution near the void volume. Attempts to restore the performance of compromised SunFire C18 columns by extending the wash step of the gradient or flushing with a higher proportion of acetonitrile content (90–100%) for several hours were ineffective.

3.2. Characterization of cyclodextrin retention by mass spectrometry

ESI-MS experiments were conducted to monitor the elution of HPCD or SBECD during the HPLC runs. This determination was not possible by UV detection due to the absence of a UV chromophore in either cyclodextrin. Fig. 4A shows the mass spectrum of HPCD obtained by infusion (60 μg/mL HPCD, 10 μL/min), which consists of singly charged molecular ions (1000–1900 amu range) that differ by 58 amu depending on the degree of hydroxypropyl substitution. The response factor of its most intense HPCD molecular ion (m/z 1411.2) is calculated from its abundance (approximately 8000 intensity units) and the sample concentration as $\sim 1.3 \times 10^5$ $(mg/mL)^{-1}$. The corresponding extracted ion chromatograms (m/z 1237.2, 1295.2, 1353.2, 1411.2, 1469.3, 1527.3, 1585.4 and 1643.4) of HPCD (2 mg/mL) analyzed by HPLC/MS are shown in Fig. 4B through I. The HPCD elutes over the course of the HPLC run as increasingly higher mass components at an intensity of up to approximately 100,000 units. No carryover was observed in subsequent consecutively injected blank and API reference standard injections, and a typical chromatographic profile was obtained by UV detection of the latter (see Fig. 2B for a representative chromatogram). Therefore, HPCD does not accumulate on the HPLC column and elutes completely over the course of an HPLC run.

By contrast, a similar evaluation of SBECD using the same HPLC conditions verified that it does not elute appreciably through the HPLC column. Fig. 5A shows the SBECD mass spectrum obtained from infusion (2 mg/mL, 10 μL/min), which consists of doubly charged molecular ions observed between 800 and 1400 amu. These are attributed to both differing degrees of sulfobutylether substitution as well as pairing with Na^+ [14]. The response factor of the most abundant SBECD molecular ion (m/z 941.8, approximately 50,000 intensity units for abundance) is $\sim 2.5 \times 10^4$ $(mg/mL)^{-1}$. The corresponding extracted ion chromatograms (m/z 753.3, 809.8, 875.5, 941.8, 1012.3, 1023.3, 1082.7 and 1093.8) obtained from the chromatographed SBECD (2 mg/mL) are shown in Fig. 5B through I. Although some SBECD elutes, it occurs only in a narrow eluting range between 53 and 58 min (the gradient wash step) and the abundance (up to approximately 1000 intensity units) is minor in comparison to the HPCD experiment. Considering that the response factor of SBECD is fivefold less than that of HPCD, and the eluting period (5 min) of SBECD is ~one-fifth of that of HPCD, an equivalent response in SBECD extracted ion chromatograms would have produced intensities of approximately 10,000. Therefore, only minor amounts of SBECD eluted from the column, while the majority is retained on the column. Furthermore, lesser levels of SBECD continue to bleed off the HPLC column within the 53–58 min time range in subsequent blank and API reference standard injections,

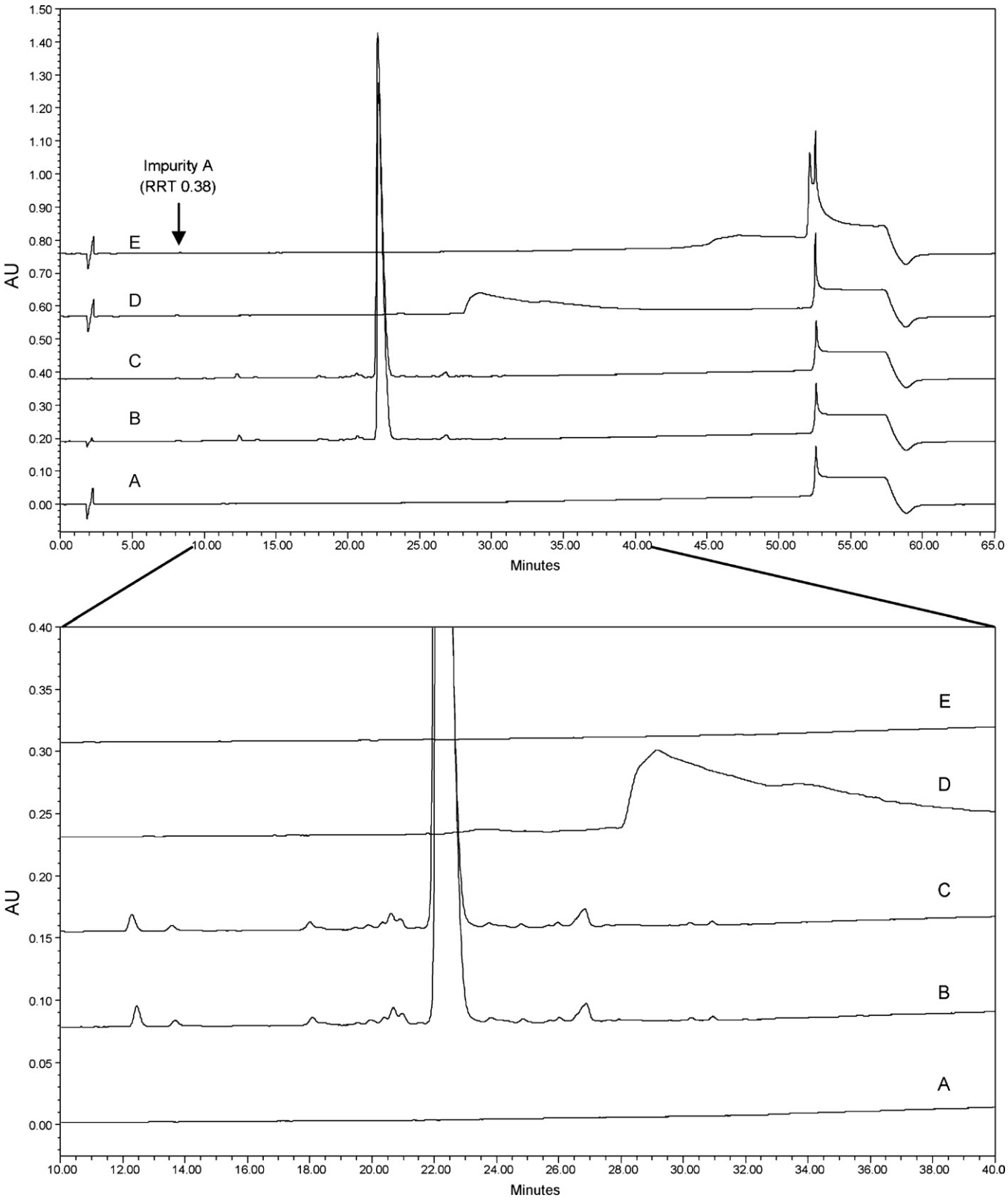

Fig. 2. Demonstration of reversed phase HPLC chromatography failure observed upon analysis of sulfobutylether-β-cyclodextrin drug product formulation. Chromatograms of blank (A), API reference standard (B) and drug product sample formulated with hydroxypropyl-β-cyclodextrin (C) precede the typical observation of the chromatography failure upon repeat injections of a drug product sample formulated with sulfobutylether-β-cyclodextrin (D and E).

and the characteristic aberrant chromatography was observed by UV detection of the latter.

The selective retention of SBECD over HPCD is likely attributed to differences in the type and extent of functional group substitution on each. These factors change the molecular symmetry of the β-cyclodextrin structure [15], which could result in differences in selectivity towards the C18 ligand. The spatial orientations of C18 ligands in β-cyclodextrins cavities have been modeled [16]. Addi-

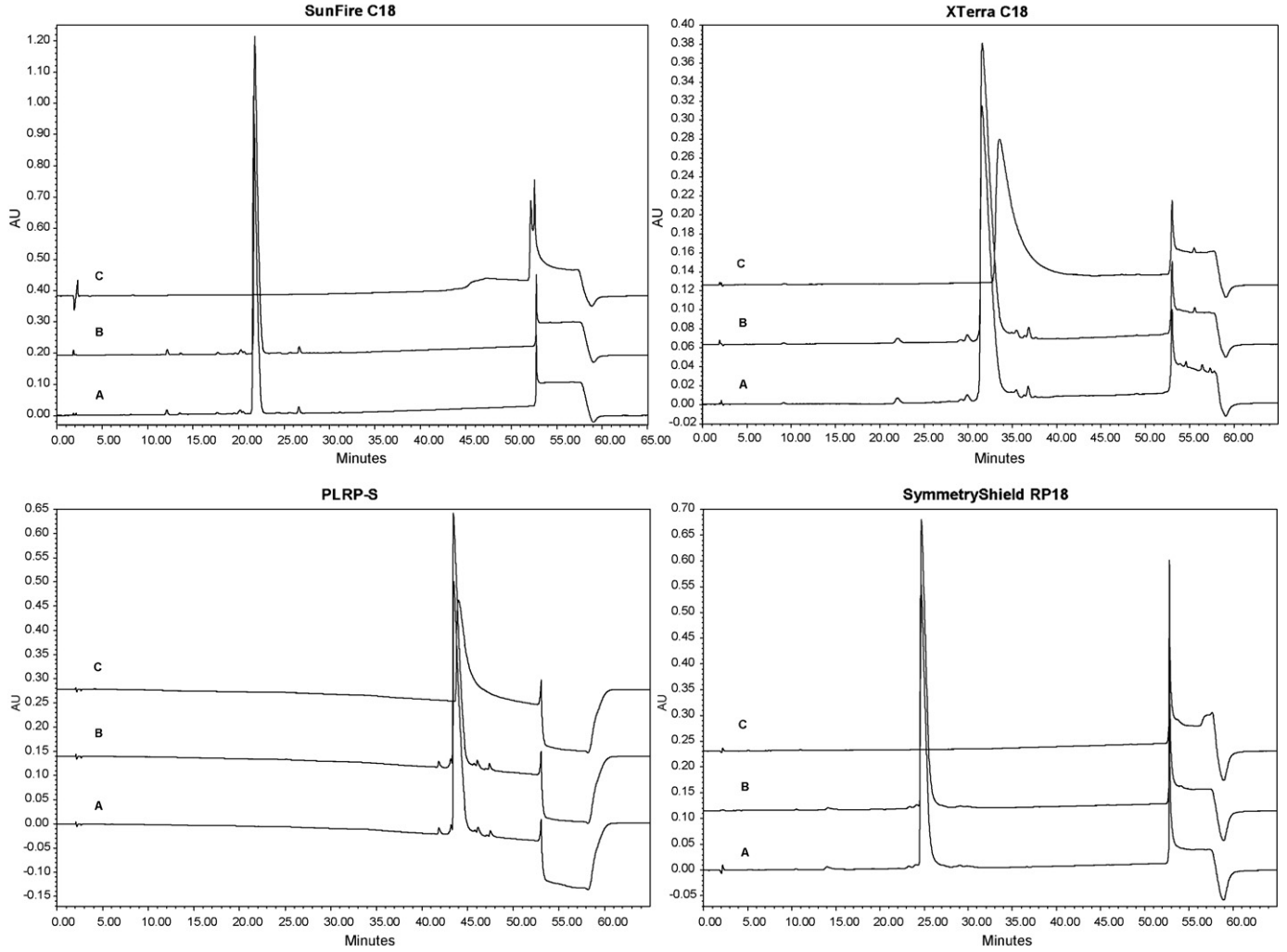

Fig. 3. Demonstration of varying degrees of reversed phase HPLC chromatography failure attributed to sulfobutylether-β-cyclodextrin in various HPLC columns (3–3.5 μm particle diameter, 150 mm × 4.6 mm). The API reference standards are shown prior to hydroxypropyl-β-cyclodextrin (A), following hydroxypropyl-β-cyclodextrin and prior to sulfobutylether-β-cyclodextrin (B), and following sulfobutylether-β-cyclodextrin (C).

tionally, the reversed phase retention properties of β-cyclodextrins on C18 were previously characterized [16,17]. Direct spectroscopic or microscopic analyses of C18 sorbents impregnated with SBECD could verify the mechanism of interaction, but were beyond the scope of this work.

The ESI-MS experiments established that SBECD accumulates on the HPLC column, resulting in a new cyclodextrin-C18 stationary phase under the HPLC conditions utilized. The retention mechanism of such a modified C18 sorbent could include ion pairing with SBECD sulfonates or inclusion complex formation, both of which

Table 3
Comparison of HPCD and SBECD impact on API peak chromatography.

Column	Injection[a]	Retention time (min)	Capacity factor[b], k	Tailing factor	Peak height (AU)	Peak width (s)
SunFire C18 (Waters, Milford, MA)	A	21.663	12.7	2.75	1,018,964	122.8
	B	21.780	12.8	2.64	1,017,633	123.2
	C	NA	NA	NA	NA	NA
PLRP-S (Polymer Laboratories, Amherst, MA)	A	43.492	21.3	5.60	523,512	140.0
	B	43.451	21.3	5.60	527,706	136.8
	C	43.989	21.6	8.95	209,434	502.4
SymmetryShield RP18 (Waters, Milford, MA)	A	24.654	14.2	4.16	550,732	157.2
	B	24.697	14.2	4.10	560,586	159.2
	C	NA	NA	NA	NA	NA
XTerra C18 (Waters, Milford, MA)	A	31.524	16.9	4.26	311,118	246.8
	B	31.564	16.9	4.23	314,245	244.8
	C	33.521	18.0	4.25	150,055	488.0

NA denotes non-availability of data, due to absence of coherent API peak.

[a] API reference standard injected prior to HPCD (A), following HPCD and prior to SBECD (B), and following SBECD (C).

[b] $k = (t_R - t_0)/t_0$, where t_0 is retention time of acetone in isocratic acetonitrile (100%, 1.00 mL/min).

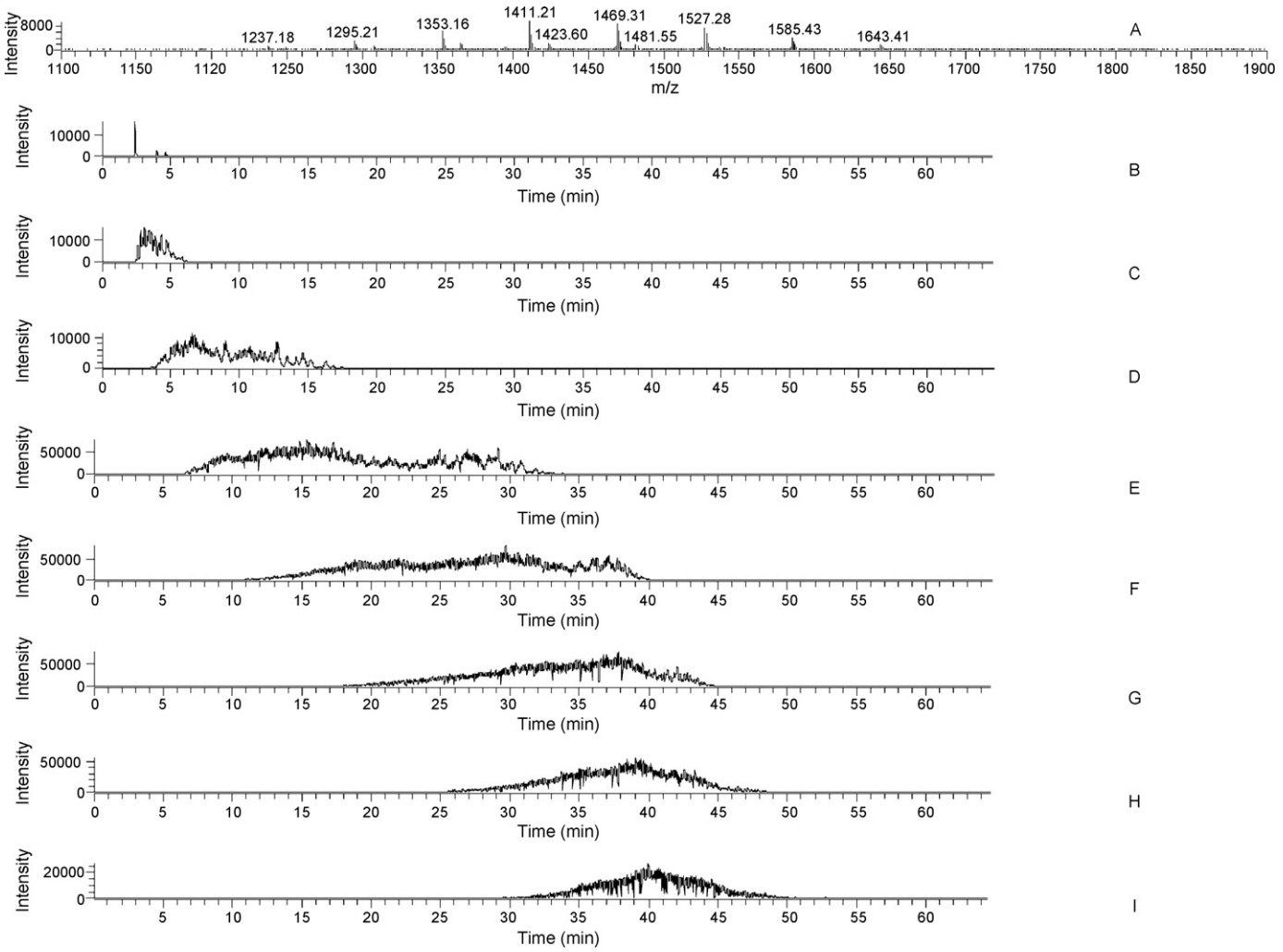

Fig. 4. Mass spectrum (ESI⁻) for infused hydroxypropyl-β-cyclodextrin (A) and corresponding total ions profiles of chromatographed hydroxypropyl-β-cyclodextrin extracted at *m/z* 1237.2 (B), 1295.2 (C), 1353.2 (D), 1411.2 (E), 1469.3 (F), 1527.3 (G), 1585.4 (H) and 1643.4 (I).

were previously discussed as advantages of using SBECD in formulation development [18] and which are nonetheless applicable in this scenario.

3.3. Effect of analyte structure on observation of aberrant chromatography

In order to determine if the chromatography failure is specific to the API, a structurally unrelated small molecule developmental drug substance (Compound X, MW ~ 400 Da) was evaluated. Compound X differs from the API in that it is not a glycopeptide, nor does it include a well-defined hydrophobic sidechain. No significant difference was observed in Compound X chromatograms produced from an untreated HPLC column (Fig. 6A) or an HPLC column in which SBECD samples were previously run (Fig. 6B). In contrast, the API reference standard exhibited typical chromatography in the same untreated HPLC column (Fig. 6C) and aberrant chromatography in the same treated HPLC column (Fig. 6D). Therefore, the chromatography failure is indeed dependent on the analyte structure itself.

The effect of structure on the observation of aberrant chromatography is also evident for the API and its structurally similar impurities. For example, API reference standard chromatograms acquired prior to an SBECD injection (Fig. 7B) and following treatment (Fig. 7D) exhibit no visible difference in the shape or retention time of the Impurity A peak (RRT 0.38). This impurity was structurally characterized and identical to the API structure except for lacking the hydrophobic sidechain (R_1; see Fig. 1). Consequently, Impurity A may lack the capability to form an effective inclusion complex with the SBECD that has accumulated on the column. Likewise, the structures of virtually all remaining impurities ≥0.10% by area lack this hydrophobic sidechain, which is consistent with the absence of additional unaffected impurity peaks.

3.4. Mitigation of chromatography failure–development and optimization of SPE procedure

An SPE sample clean-up procedure was developed with the goals of removing SBECD in a highly selective manner, producing high recoveries of the API and its impurities and minimizing any impact to the chromatographic profile. Consequently, no modification to the sample solution pH or its components was pursued. Although a cyclohexyl SPE procedure was developed previously to isolate SBECD by a reversed phase mechanism [19], the presence of a hydrophobic sidechain on the API and the potential for low recoveries precluded its use for this application. Instead, an anion-exchange mechanism was pursued to take advantage of the multiple sulfonate groups in SBECD (1–12 groups [1], $pK_a \sim -1$) and the absence of anionic structures on the API in acidic sample diluent (0.05% formic acid,

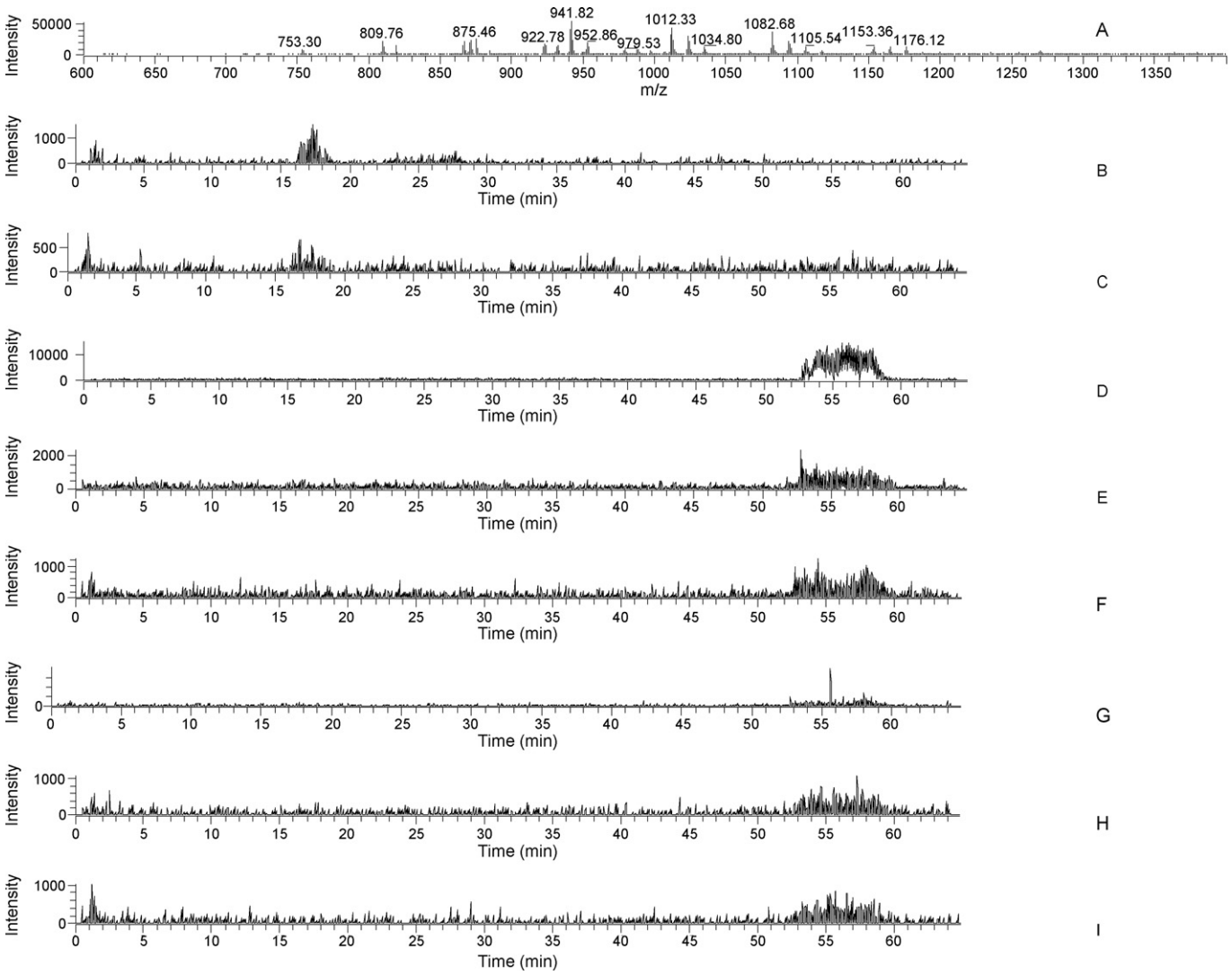

Fig. 5. Mass spectrum (ESI⁻) for infused sulfobutylether-β-cyclodextrin (A) and corresponding total ion profiles of chromatographed sulfobutylether-β-cyclodextrin extracted at *m/z* 753.3 (B), 809.8 (C), 875.5 (D), 941.8 (E), 1012.3 (F), 1023.3 (G), 1082.7 (H) and 1093.8 (I).

pH ∼ 1.8). The selection of quaternary amine SPE sorbent was based on its previous utilization in combination with evaporative light scattering detection for the direct analysis of SBECD [20].

The quaternary amine SPE procedure was optimized with respect to API recovery, impurity recovery and HPLC chromatography considerations. The acetonitrile content of sample solutions was varied from 2 to 20% (each in the presence of 0.05% formic acid) to evaluate its effect on a possible inclusion complex comprised of bound (i.e., ion paired) SBECD with the API. Near quantitative API recoveries of 98.6 and 101.4% were obtained using 15 and 20% acetonitrile, respectively. Also, the API recoveries were directly proportional to the acetonitrile content (Fig. 8A), which is consistent with the destabilization of the host–guest interaction by increasing the solvent strength. However, increasing the acetonitrile content above 10% resulted in the absence or diminished recovery of the Impurity A peak (RRT ∼ 0.38) and broadened impurity peaks in the chromatographic profile.

Since the 10% acetonitrile sample diluent produced acceptable recoveries of the API (94.9%) and of selected impurities including Impurity A (108–117%; see Table 4), this composition was adopted as the key parameter in the optimized SPE procedure. Note that impurity recoveries were determined as percentages of the relevant impurity peaks in the API reference standard. Additionally, an unknown artifact peak (RRT 1.21) was observed in the 10% acetonitrile sample diluent chromatogram, which also increased with diminishing acetonitrile content as shown in the 2 and 5% acetonitrile levels. Although using a sample diluent with 15% acetonitrile would have prevented the occurrence of this unknown artifact peak, it was still very minor in the adopted 10% acetonitrile preparation and sacrificing the recovery of key impurities such as Impurity A was not feasible.

Table 4
Recoveries of select impurities in 100% API level sample using optimized solid-phase extraction procedure ($n = 5$ replicates).

Peak	% w/w	Recovery (%)	% R.S.D.
Impurity A (RRT 0.38)	0.12	112.9	10.21
Impurity B (RRT 0.56)	0.82	116.8	0.75
Impurity C (RRT 1.38)	0.11	108.2	6.43

The optimized SPE procedure was conducted well within the loading capacity of the quaternary amine sorbent, but is not particularly robust to variances in SBECD content. Solutions containing 0.8–3.2 mg/mL of SBECD (equivalent to 50–200% of nominal) and nominal levels of mannitol (0.6 mg/mL) and API (0.5 mg/mL) that

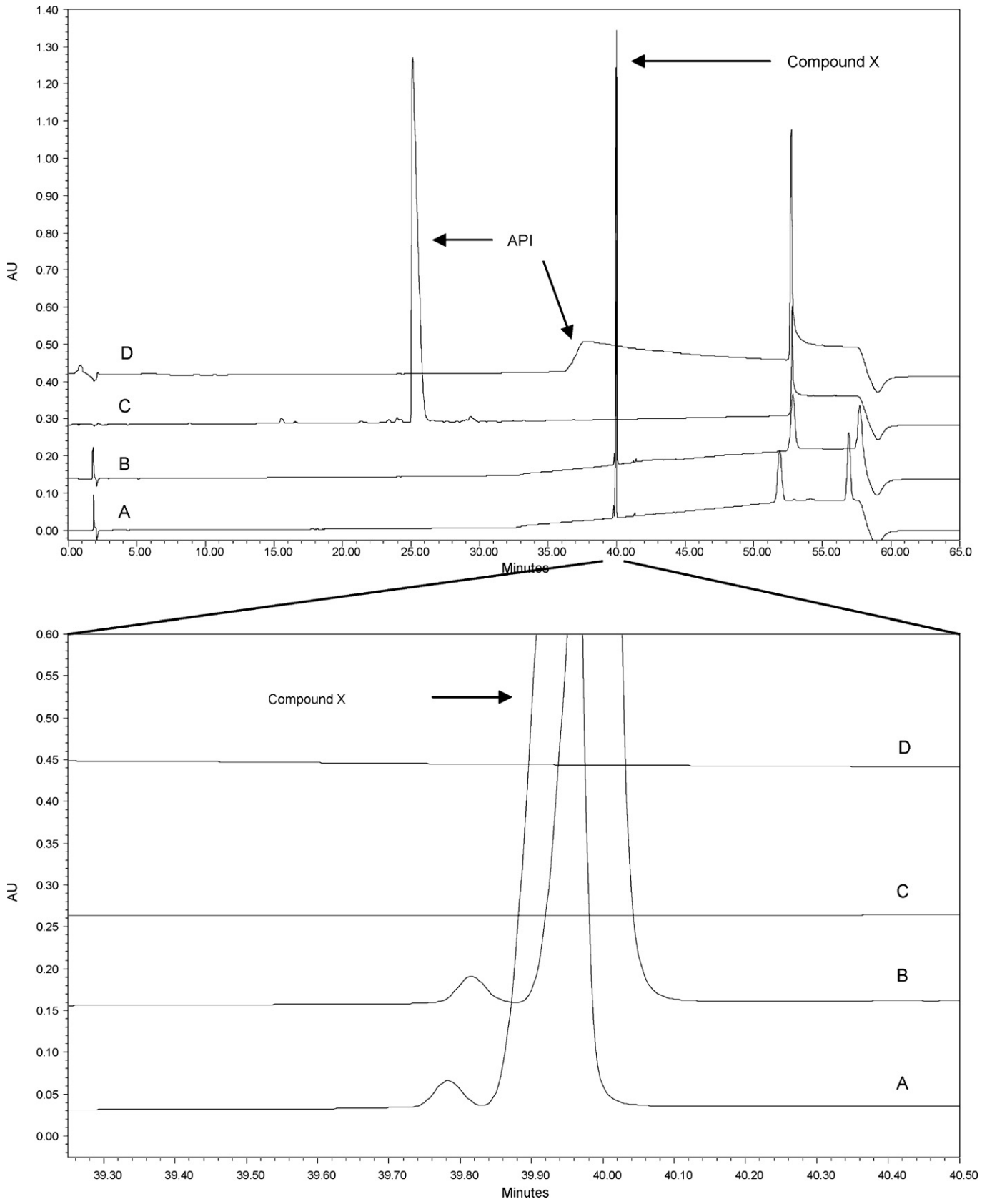

Fig. 6. Demonstration of reproducible chromatography for Compound X in SunFire C18 columns through which sulfobutylether-β-cyclodextrin samples were not previously run (A) and were previously run (B). These chromatograms are compared to API reference standards run on the same columns, exhibiting typical chromatography (C) and the chromatography failure (D).

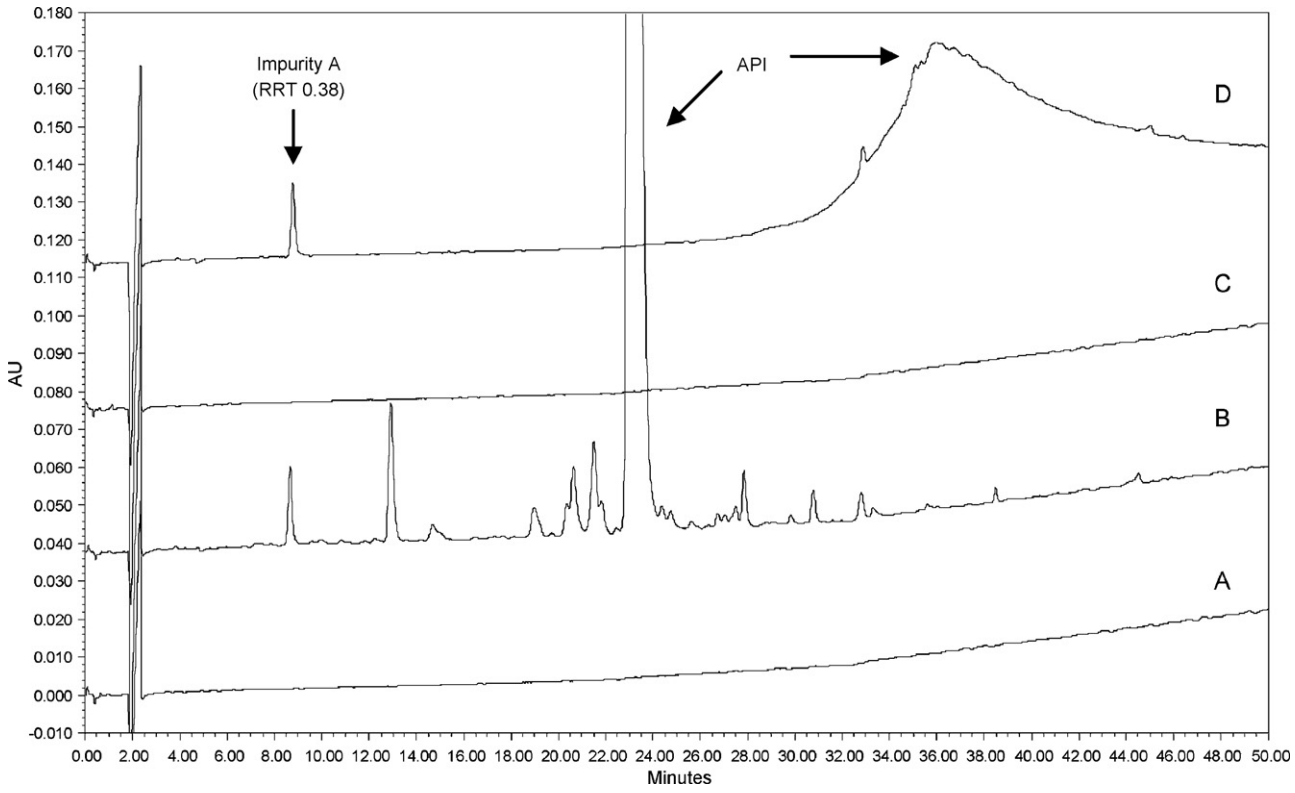

Fig. 7. Demonstration of unchanged Impurity A chromatography following SBECD exposure to SunFire C18 column. Expanded chromatograms of blank (A) and API reference standard (B) precede sulfobutylether-β-cyclodextrin only (C) and a repeat injection of an API reference standard (D).

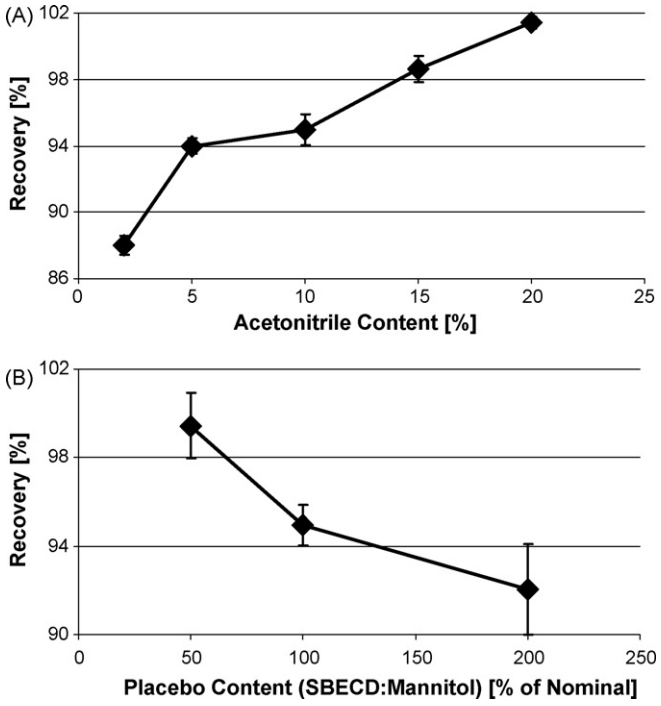

Fig. 8. Effect on API recovery ($n = 5$) by varying acetonitrile content in sample diluent (A). Effect of API recovery ($n = 3$) by varying the content of excipients including sulfobutylether-β-cyclodextrin (B). Variability (as % R.S.D.) is indicated as error bars for each data point.

were treated by SPE each produced typical chromatographic profiles. Therefore, the actual SBECD loading capacity of the quaternary amine SPE cartridges is at least twice that present in a typical drug product sample. However, a trend of inversely proportional API recovery with SBECD content was observed (Fig. 8B), which is attributed to the increased potential for API-SBECD interactions (e.g., inclusion complex, ion pairing) as more SBECD is bound to the quaternary amine sorbent. This would be an inherent limitation to any SPE procedure applied for removing SBECD, which could be overcome by using a sample diluent with higher organic content or simply ensuring that adequate manufacturing controls are in place to reduce the variability of SBECD content in drug product samples.

Table 5
Recoveries of API (0.10–150% of 0.2 mg/mL nominal) in presence of nominal excipient using optimized solid-phase extraction procedure ($n = 5$ replicates).

API level (%)	Recovery (%)	% R.S.D.
0.10	91.0	19.83
1	93.4	3.80
25	97.8	0.48
50	95.3	1.32
100	94.9	0.92
150	95.5	0.45

In order to qualify the optimized SPE procedure, linearity and accuracy were evaluated between 0.10 and 150% of the nominal API concentration (0.2 mg/mL in final prepared sample). The least squares regression plot ($y = 267{,}185x + 30{,}181$) exhibited an excellent linear fit, as evidenced by its coefficient of determination (r^2, 0.999). Recovery and precision data are summarized in Table 5. Acceptable API recoveries were obtained in the accuracy evaluations, ranging from 91 to 98%. The precision at the nominal range (i.e., 25–150% API level) was up to 1.3% R.S.D., which is excellent in consideration that this method includes a sample clean-up step. The precision at the impurity level (i.e., 1% spike level and below) was up to 20% R.S.D., which is typical of the variability obtained near the limit of quantitation. Therefore, the optimized SPE procedure is effective for determining the API and its impurities.

4. Conclusions

A novel reversed phase HPLC chromatography failure attributed to SBECD has been characterized. The mechanism of the chromatography failure includes the modification of reversed phase column sorbents by the accumulation of SBECD, followed by its preferential interaction with the API and the observation of a broad, late-eluting API peak. The retention of SBECD is likely to occur for any reversed phase HPLC application employing strongly acidic mobile phases (pH < 2), although this could go unnoticed for analytes that do not interact appreciably with SBECD. A column regeneration procedure was developed to restore the performance of reversed phase HPLC columns that have been compromised by SBECD. The chromatography failure was addressed by developing an effective, optimized solid-phase extraction clean-up procedure to selectively remove SBECD from a drug product sample solution. While this procedure is specific to the API and to the HPLC analysis method employed, a similar development and optimization process would apply to any drug product containing SBECD.

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