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Comparison of drug substance impurity profiles generated with extended length columns during packed-column SFC

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Abstract

The current study assesses the effect of extending column length during gradient packed column sub/supercritical fluid chromatography (PCSFC) experiments on the detection of known and unknown impurities in a drug substance sample. Quantitative drug substance impurity profiles were generated and compared using multiple column PCSFC and HPLC conditions. Also, chromatographic figures of merit were estimated and compared for components of a standard mixture during PCSFC experiments, which used one column, four columns, and six columns in series.

Keywords: Packed column sub/supercritical fluid chromatography; Impurity profile; Drug sunstance analysis

1. Introduction

During drug development and manufacture, purity determinations for GMP lots of drug substance are typically implemented with high-resolution chromatographic methods, usually HPLC. One of the most important aspects of chromatographic purity determinations is the capacity of the procedure to resolve synthetic process impurities, which are often structural analogues or homologues of the 'parent' compound. Resolving capabilities of chromatographic procedures are critical during early stages of drug development, when the numbers and levels of unknown impurities in drug substance lots are not well characterized and the procedures provide an assessment of the suitability of the drug substance lot for use in safety studies and clinical trials. Resolving capabilities are also important for measuring the equivalency of drug substance lots synthesized with modified manufacturing processes or at different manufacturing sites.

Numerous recent reports have demonstrated the applicability of packed-column sub/supercritical fluid chromatography (PCSFC) to the analysis of drug substance and drug product analyses ([1–8], [9–15]). Gyllenhall et al. recently described applications of PCSFC completed in support of drug development ([16]). Our recent studies have concerned evaluating capabilities originally ex-

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pressed in a report by Berger and Wilson during 1994 ([17]), which demonstrated that PCSFC experiments could be completed with several packed-columns in series (due to the low viscosity of carbon dioxide mobile phases), creating an extended length column. This study demonstrated that PCSFC experiments with extended length columns could generate chromatographic efficiencies (reflected as theoretical plate counts in excess of 200,000 for ten, 20 cm columns used in series) not obtainable during packed-column HPLC experiments.

We have examined the effect of extending the length of packed-columns during PCSFC experiments (using multiple 25 cm columns in series) on the detection and resolution of impurities detected in lot of SC-65872, which is currently undergoing clinical trials. Structures for SC-65872 and related compounds are shown in Fig. 1. The basis of our interest has been that extending the column length is a straightforward, quick approach to improving the separation power of a procedure. We wanted to evaluate the use of this approach in the context of a pharmaceutical analysis problem, due to the potential for applicability to a variety of analysis problems in our laboratories.

We developed a single-column, gradient PCSFC procedure to provide resolution for SC-65872 and a mixture of related compounds. The same mixture of compounds was used to optimize the existing HPLC procedure for SC-65872 drug substance analysis. We determined the effect of extending the column length (using one, four, and six columns in series) on the SC-65872 standard mixture separation by estimating chromatographic figures of merit for the single and multiple column PCSFC chromatograms. In addition, we evaluated the effect of extending the column length on the numbers and levels of impurities detected in a lot of SC-65872 synthesized during early drug development activities and compared the PCSFC impurity profiles to the profiles generated with a previously developed gradient HPLC procedure. The same temperature and modifier gradients were used for the single and multiple column PCSFC experiments.

2. Experimental

2.1. Materials and reagents

Samples of SC-65872 and related compounds were prepared by the Chemical Sciences Department of Searle Research and Development. The HPLC assay value for the SC-65872 lot used in the current study was $>98\%$. All HPLC mobile phase components were reagent-grade and used as purchased. All SFC experiments were completed

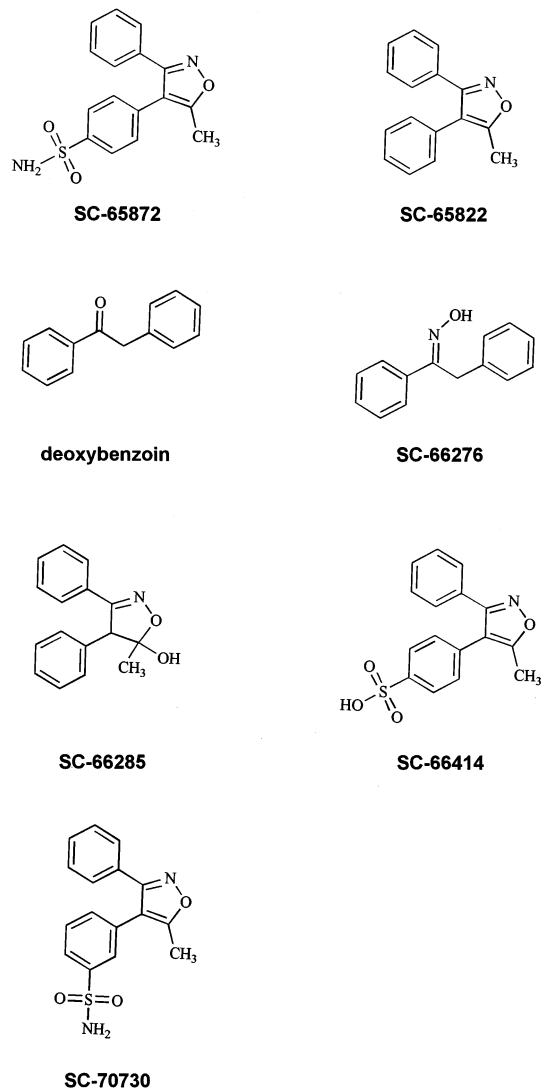


Fig. 1. Structures for SC–65872 and related compounds.

with supercritical fluid grade carbon dioxide purchased from Scott Specialty Gases. Modifiers for SFC experiments were HPLC grade solvents.

2.2. SFC experiments

All SFC experiments were completed with a prototype Berger Instruments Packed-Column Supercritical Fluid Chromatograph (Newark, DE) and a Model 1050 Hewlett Packard photodiode array detector equipped with a high pressure cell. The SFC was also equipped with a manual Valco injection valve. Optimized conditions were as follows: columns-Supelcosil LC- CN (250 mm × 4.6 mm i.d.; 5 μ particle size); mobile phase flow-rate-3.0 ml/min; injection volume-10 μl; detection wavelength-240 nm; outlet pressure 120 bar. Mobile phase A for the gradient PCSFC procedure was carbon dioxide. Mobile phase B was methanol with 0.5% (vol/vol) isopropylamine. The mobile phase program was as follows: initially 98% mobile phase A (no hold); ramp at 1% per min up to 30% mobile phase B. A temperature gradient was also administered: initial temperature − 32°C; hold for 7 min then ramp at 0.8°C to 45°C. Each Supelco LC-CN column was evaluated with the SC-65872 standard mixture prior to use in series with other columns. Columns were connected in series with 5 cm. long sections of 0.01 inch i.d. stainless steel tubing.

For the standard mixture chromatograms, the SC-65872 concentration was 0.25 mg/mL. Related compound concentrations were approximately 5% of the SC-65872 concentration. For external standard quantitation measurements the SC-65872 sample concentration was 12.5 mg/mL. All SC-65872 standards and samples were dissolved in acetonitrile:water:methanol (1:1:1; vol/vol/vol). The external standard impurity levels were calculated with a 0.5% (wt/wt) SC-65872 external standard, using area responses.

2.3. HPLC experiments

All HPLC experiments were completed with a system comprised of a Hewlett-Packard 1050 liquid chromatograph equipped with a Kratos 783 variable wavelength UV-visible detector. Reverse-phase HPLC conditions were as follows: column-Supelco Diphenyl LC-DP (250 mm × 4.6 mm i.d.; 5 μ particle size); mobile phase flow-rate-1.0 mL/min; injection volume-15 μL; detection wavelength-215 nm; column temperature-40°C. Each liter of aqueous mobile phase A was comprised of 10% (v/v) acetonitrile and 90% (v/v) 20 mM phosphate buffer, pH 3. Mobile phase B was comprised of 75% (v/v) acetonitrile and 25% (v/v) 20 mM phosphate buffer, pH 3. The mobile phase gradient program was as follows: 0 min-70% mobile phase A, 30% mobile phase B; 25 min-55% mobile phase A, 45% mobile phase B; 43.3 min-100% mobile phase B; 44.3 min-70% mobile phase A, 30% mobile phase B; 63 min-70% mobile phase A, 30% mobile phase B. The SC-65872 sample concentrations were approximately 1067 mcg/mL. The external SC-65872 standard used for quantitation of the impurities was approximately 5.3 mcg/mL or 0.5% of the sample SC-65872 concentrations. The sample diluent was 30% (v/v) acetonitrile and 70% (v/v) water. Sample impurity levels were calculated using the average SC-65872 peak area response factor calculated for six bracketing injections of the 0.5%-level SC-65872 external standard.

2.4. Other procedures

Chromatographic peak integration and data management were completed with the PE Nelson Turbochrom 3 Multitasking Data System. Chromatographic parameters in Table 1 were also calculated by Turbochrom, using the following equations:

$$T = \frac{W_{0.05}}{2 \times f}$$

T is the tailing factor
$W_{0.05}$ is the peak width at 5% peak height
f is the width (time) between the peak maximum and the front edge of the peak at 5% of the peak height

$$R_{p2} = \frac{2(T_{p2} - T_{p1})}{W_{p2} + W_{p1}}$$

R_{p2} is the resolution of peak 2
T_{p1} is the retention time of peak 1

Table 1
Chromatographic figures of merit generated with PCSFC and HPLC methods

		PCSFC				HPLC
		One column	four columns	six columns		one column
Tailing factor	SC-66276	1.47	1.26	1.10	SC-66285	0.79
	SC-66285	1.64	1.22	1.04	SC-66276	1.01
Resolution (from previous peak)	SC-66276	8.87	10.39	19.34	SC-66285	1.99
	SC-66285	4.49	5.31	10.11	SC-66276	8.52
Ret. (min.)	SC-66276	2.4	8.0	11.2	SC-66285	19.3
	SC-66285	3.0	9.0	12.3	SC-66276	24.1
Ret. Range (min.)*		9.0	12.4	13.4		30.2

* For all peaks in the standard mixture

T_{p2} is the retention time of peak 2
W_{p1} is the width at the base of peak 1
W_{p2} is the width at the base of peak 2

3. Results and discussion

3.1. Initial method development / single column PCSFC standard mixture chromatograms

Our initial method development activities with PCSFC concerned developing conditions, which provided resolution of SC-65872 and the related compounds shown in Fig. 1. These compounds include all of the synthetic process intermediates as well as a process impurities, SC-66414 and SC-70730. Compound SC-66414 is an immediate synthetic precursor and has been shown to be a degradation product of SC-65872 during forced degradation studies at elevated temperatures as well as a trace level synthetic process impurity. The standard mixture comprised of the compounds in Fig. 1 was also used to optimize the HPLC procedure used to analyze SC-65872 drug substance samples produced during early development activities.

Method development studies focussed on several factors, including the organic modifier choice, the use of isopropylamine as a constituent in the modifier, the temperature gradient, and column type. Two critical choices shown during method development were the choice of organic modifier and the use of isopropylamine in the modifier. Isopropylamine has been previously characterized as an effective modifier for PCSFC separations ([18]). It was found that peak symmetry improved when methanol was used rather than acetonitrile. Also, the use of isopropylamine at the 0.5% level in the methanol produced narrower bands and resulted in improved peak symmetry for SC-66414, which was asymmetric without the use of isopropylamine.

Fig. 2A shows a chromatogram recorded for the SC-65872 standard mixture using optimized, gradient PCSFC conditions. Baseline resolution of all the components in the standard mixture was achieved in 11 min, with the exception of SC-70730 and SC-65872, which were partially resolved. Fig. 2B shows the chromatograms of the standard mixture achieved with the gradient HPLC conditions that have been utilized for GLP testing of SC-65872 drug substance samples. Baseline resolution of the components in the standard mixture was achieved in about 35 min. The order of elution for the standards recorded with the procedures were the inverse of each other, with the exception of deoxybenzoin and SC-65822.

3.2. PCSFC standard mixture chromatograms with four, six, and eight columns in series

Our intention during this study was to utilize several packed colums in series to generate extended column lengths during PCSFC and to evaluate the effect of increasing column length on standard mixture chromatograms and synthetic

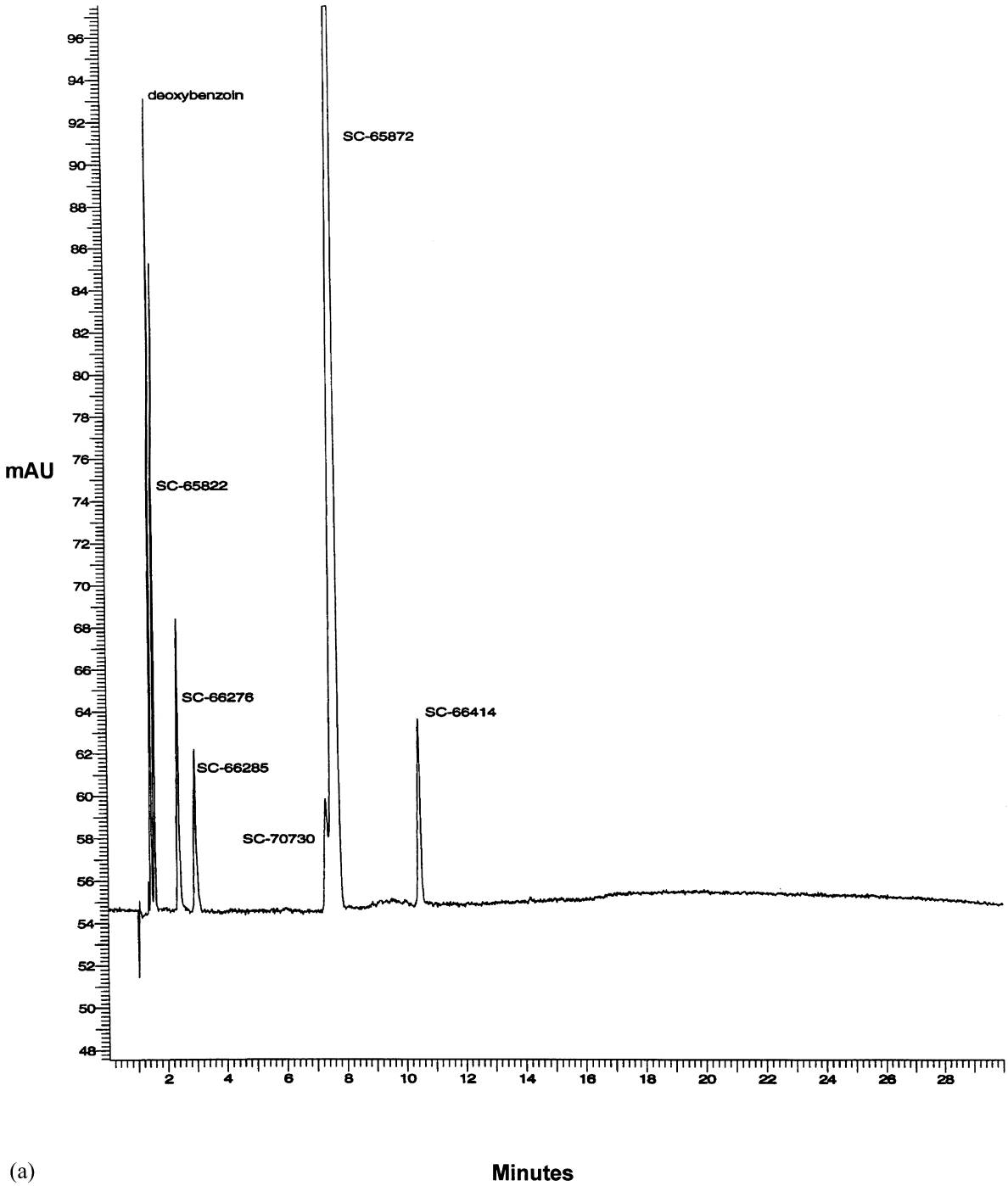
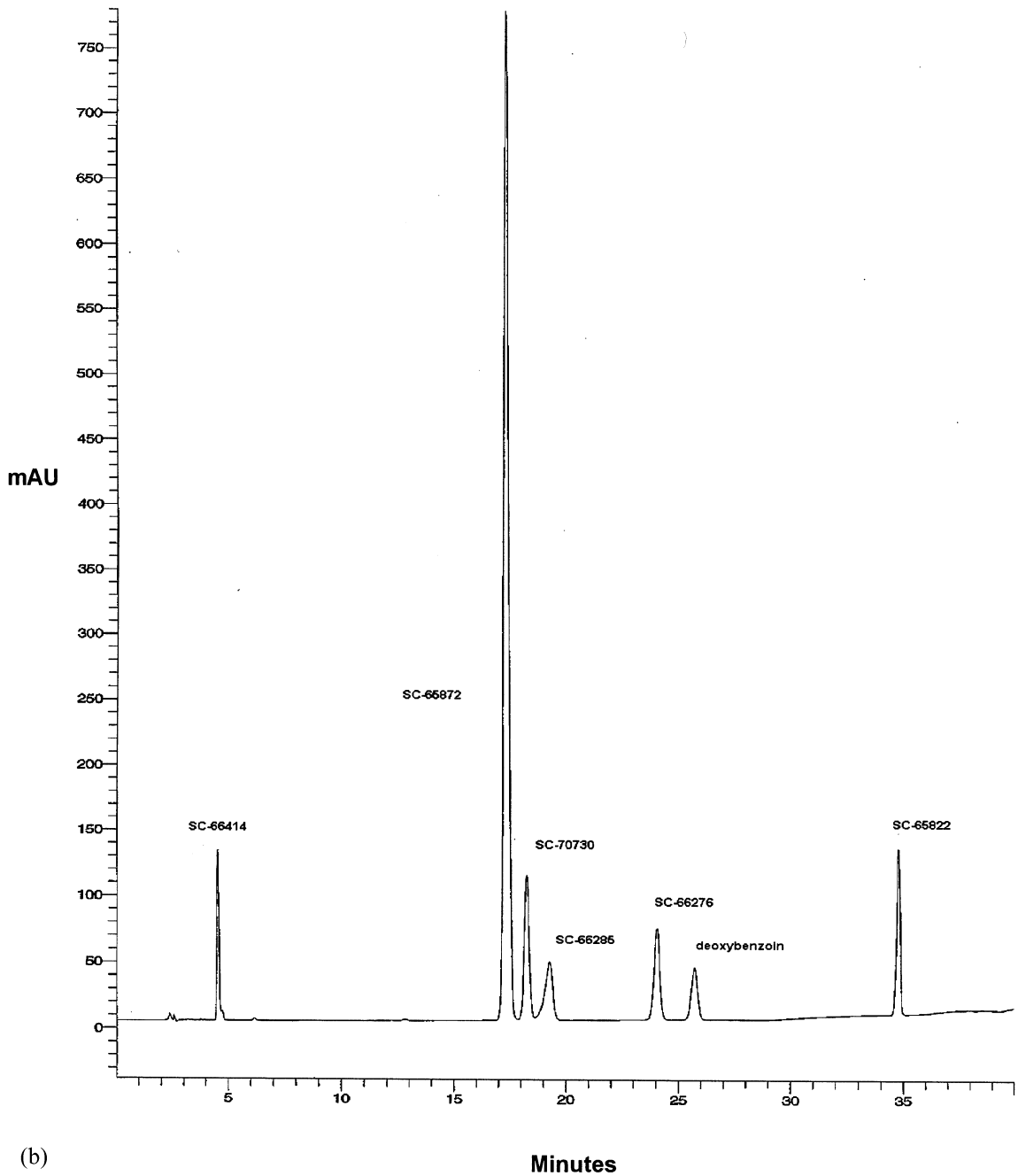


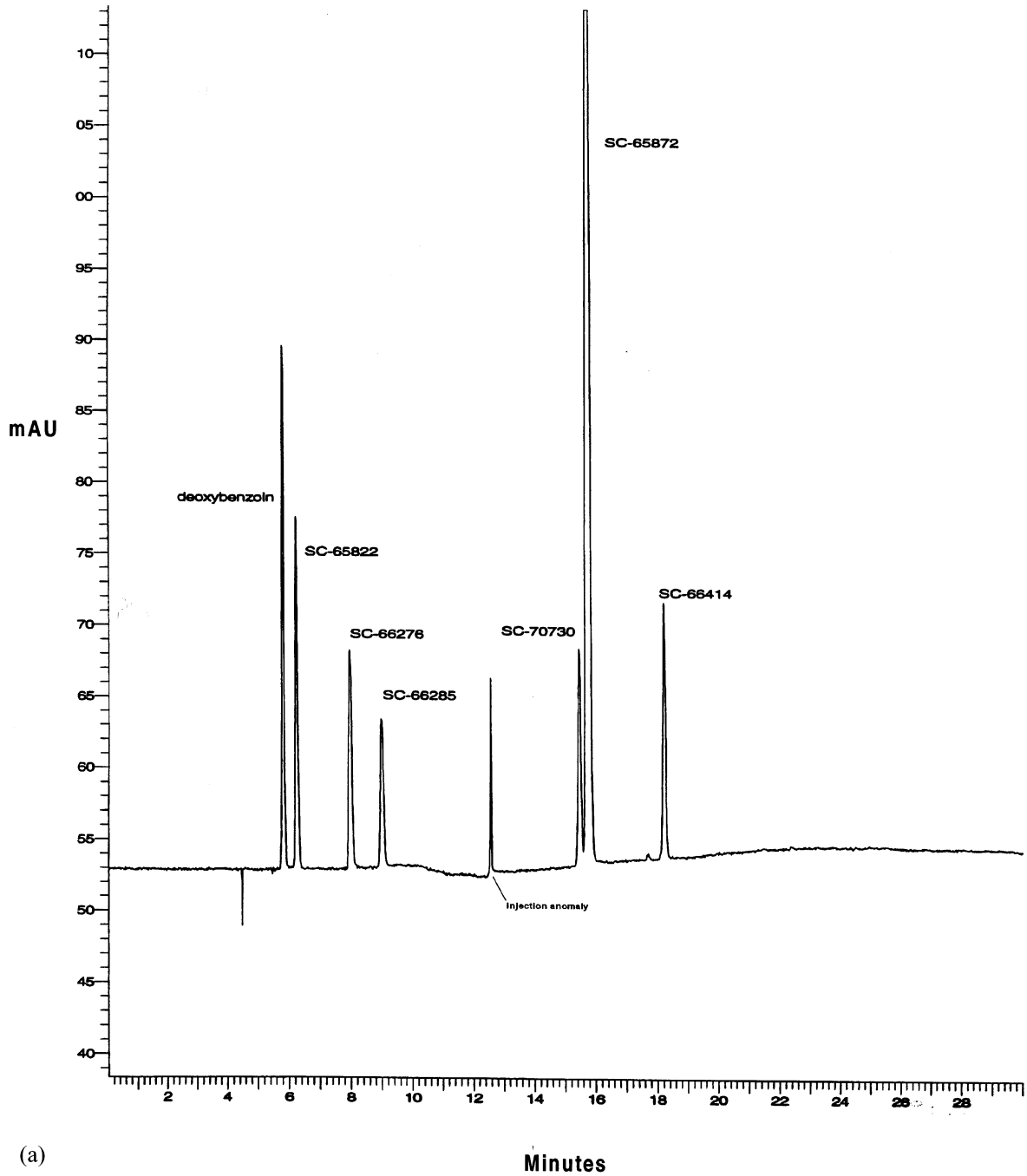
Fig. 2. SC-65872 standard mixture chromatogram: A) PCSFC; B) HPLC.

process impurity profiles. Fig. 3A-C show SC-65872 standard mixture chromatograms utilizing four, six, and eight columns in series. The chromatograms exhibit improved resolution as the column length is increased. It should be noted that baseline deflections were observed in re-



(b)

Fig. 2. (*Continued*)



(a)

Fig. 3. SC-65872 standard mixture chromatograms generated with multiple column PCSFC: A) four columns; B) six columns; C) eight columns.

sponse to blank injections during PCSFC with multiple columns at retention times of approximately 12.5 min 16.2 min and 19.0 min (note chromatograms in Fig. 3A,3B, and 3C). We attributed the deflection to differences in sample injection solvent and mobile phase composition.

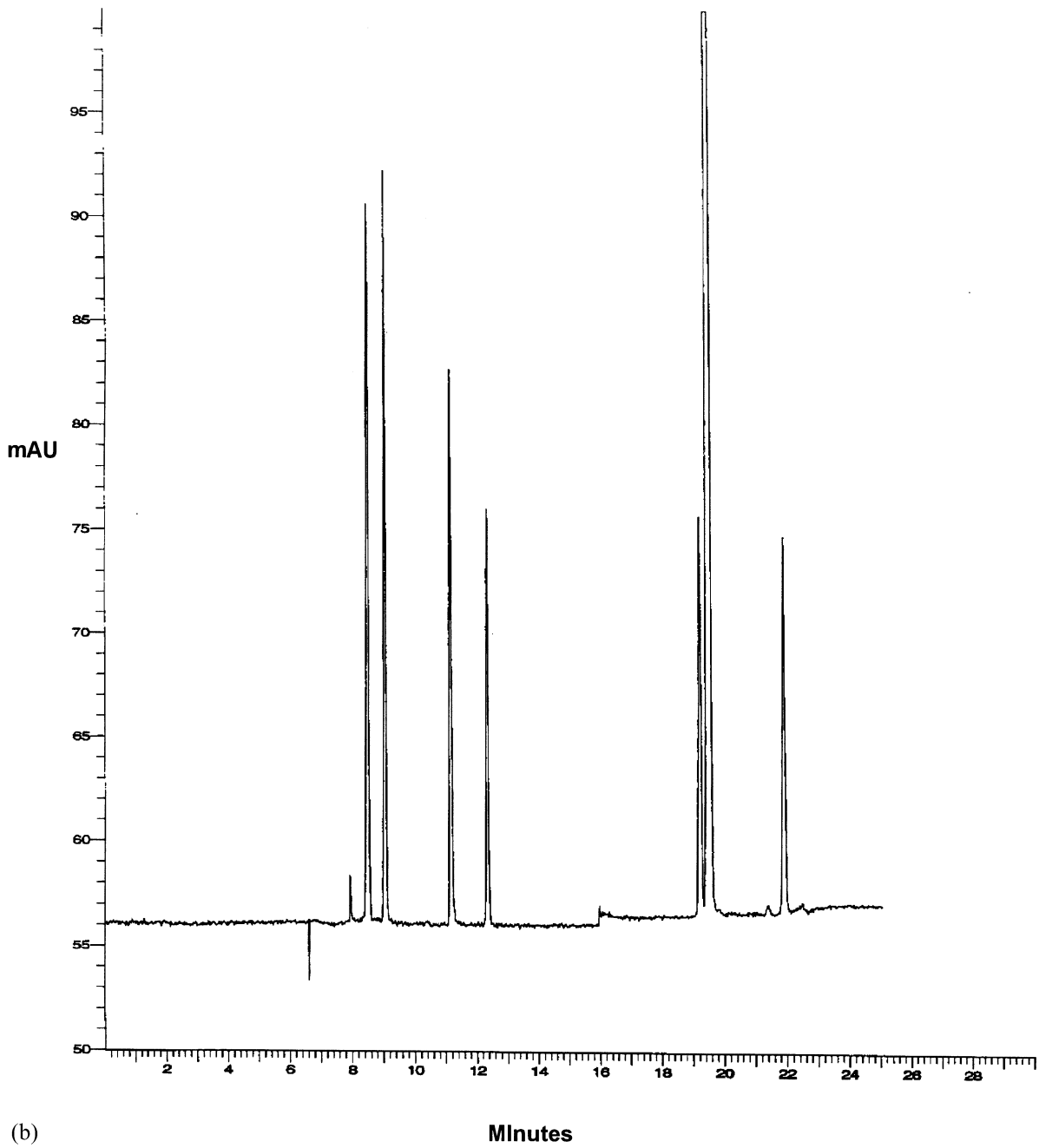


Fig. 3. (*Continued*)

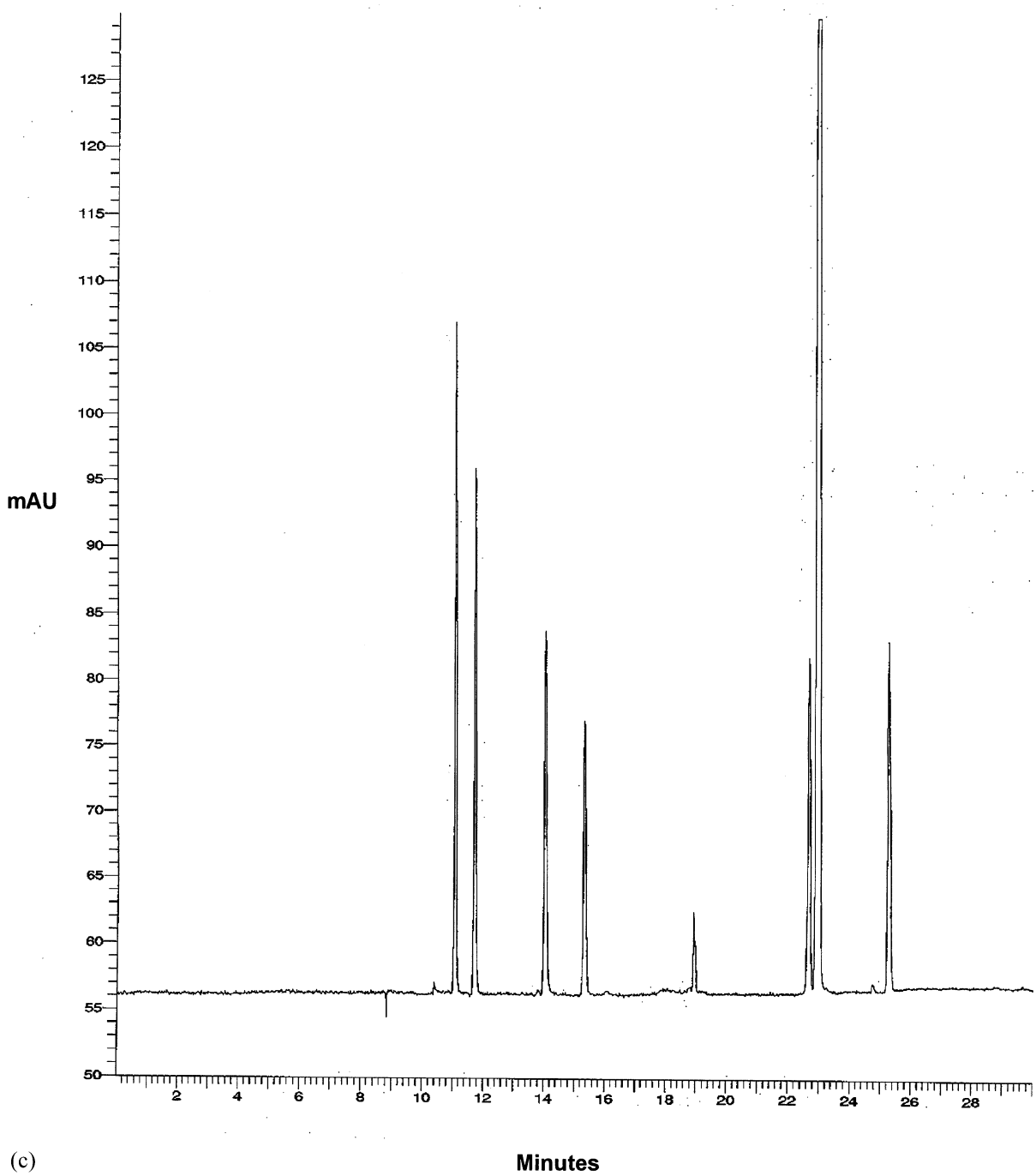


Fig. 3. (*Continued*)

It represents a potential interference to the determination of impurities in the drug substance sample.

Table 1 summarizes three chromatographic figures of merit generated with PCSFC for two of the compounds in the SC-65872 standard mixture,

SC-66276 and SC-66285. These include tailing factors, resolution factors, and retention time. Values are summarized for PCSFC chromatograms generated with one, four, and six columns in series. For comparison, figures of merit recorded for the same compounds using the gradient HPLC method are also summarized. Tailing factor values observed with PCSFC decreased from ~ 1.5 to ~ 1.0 as the number of columns in series increased from one to six, reflecting improved peak symmetry. Improved tailing factors with longer columns suggests that improvements in this figure of merit are due to extra-column band broadening effects. Typically, asymetrical peak shape due to extra-column effects can be improved by using a longer column. Peak tailing parameters from one-column HPLC experiments were slightly better than values observed with multi-column PCSFC. Resolution factors for SC-66276 and SC-66285 increased as PCSFC column length increased from one to six and were greater than the values estimated for one-column HPLC experiments. The favorable comparison of resolution factors (for PCSFC relative to HPLC) is significant when it is considered that the retention times for SC-66276 and SC-66285 recorded during PCSFC were lower than during HPLC experiments. The retention time range (difference between the earliest and latest eluting standards) with the PCSFC conditions increased from 9 min to 13.4 min as the column length increased. The range of retention times observed with the HPLC conditions was 30.2 min.

3.3. SC-65872 impurity profiles generated with PCSFC and HPLC

To provide further characterization of the PCSFC method, quantitative impurity profiles were generated for a recently synthesized SC-65872 drug substance lot. The intent was to provide comparison of the profiles as column length increased and to compare results with those observed with HPLC. Due to several factors, we utilized relatively high injection levels of 125 μg to generate the impurity profiles with PCSFC. We wanted to exaggerate the profile to make comparisons more meaningful. Also, the chromatography conditions utilized with the PCSFC procedure yielded relatively low sensitivity. The photodiode array detector utilized with PCSFC provided less than optimum sensitivity and the use of isopropylamine as a mobile phase modifier additive dictated the use of a relatively high detection wavelength (240 nm), due to baseline change during administration of the gradient. Fig. 4 provides a comparsion of the sensitivity of the PCSFC and HPLC conditions for an 8 μg injection of SC-65872. The area response for the SC-70730 impurity peak is 8-9 times greater with the HPLC system, which utilized detection at 215 nm.

Fig. 5A-C show high sensitivity chromatograms recorded with the gradient PCSFC conditions, with single and multiple columns in series. Detected impurities totalled 14, 16, and 21 with one, four, and six column PCSFC conditions, respectively. Also shown in Fig. 5A-C are the chromatograms for a 0.5% external standard. Fig. 6 shows the impurity profile observed with the HPLC conditions. The injection quantity of 16 μg for the HPLC chromatogram generated the same signal to noise as the 125 μg injection generated with the PCSFC conditions (note Fig. 4). Detected impurities with HPLC totalled 21, which was the same number of impurities observed with PCSFC using six columns in series. A signal to noise of approximately three was required to qualify a peak as significantly different from baseline noise during PCSFC and HPLC experiments.

Quantitative impurity results observed with the one and six column PCSFC methods and the HPLC method are summarized in Table 2. The one column and six column PCSFC conditions resolved 10 and 11 impurities at levels $\geq 0.01\%$, respectively. The HPLC conditions yielded twelve impurites $\geq 0.01\%$. Impurity totals for one column PCSFC, six column PCSFC, and HPLC (for impurities $\geq 0.01\%$) were 0.28, 0.53, and 1.06%, respectively. A significant portion of the difference in the impurity totals for HPLC and six column PCSFC was the discrepency in the quantitated level for SC-70730 (0.22 and 0.56% for PCSFC and HPLC, respectively). Since a 0.5% (wt/wt) standard of SC-65872 was used as an external standard for quantitating impurity levels,

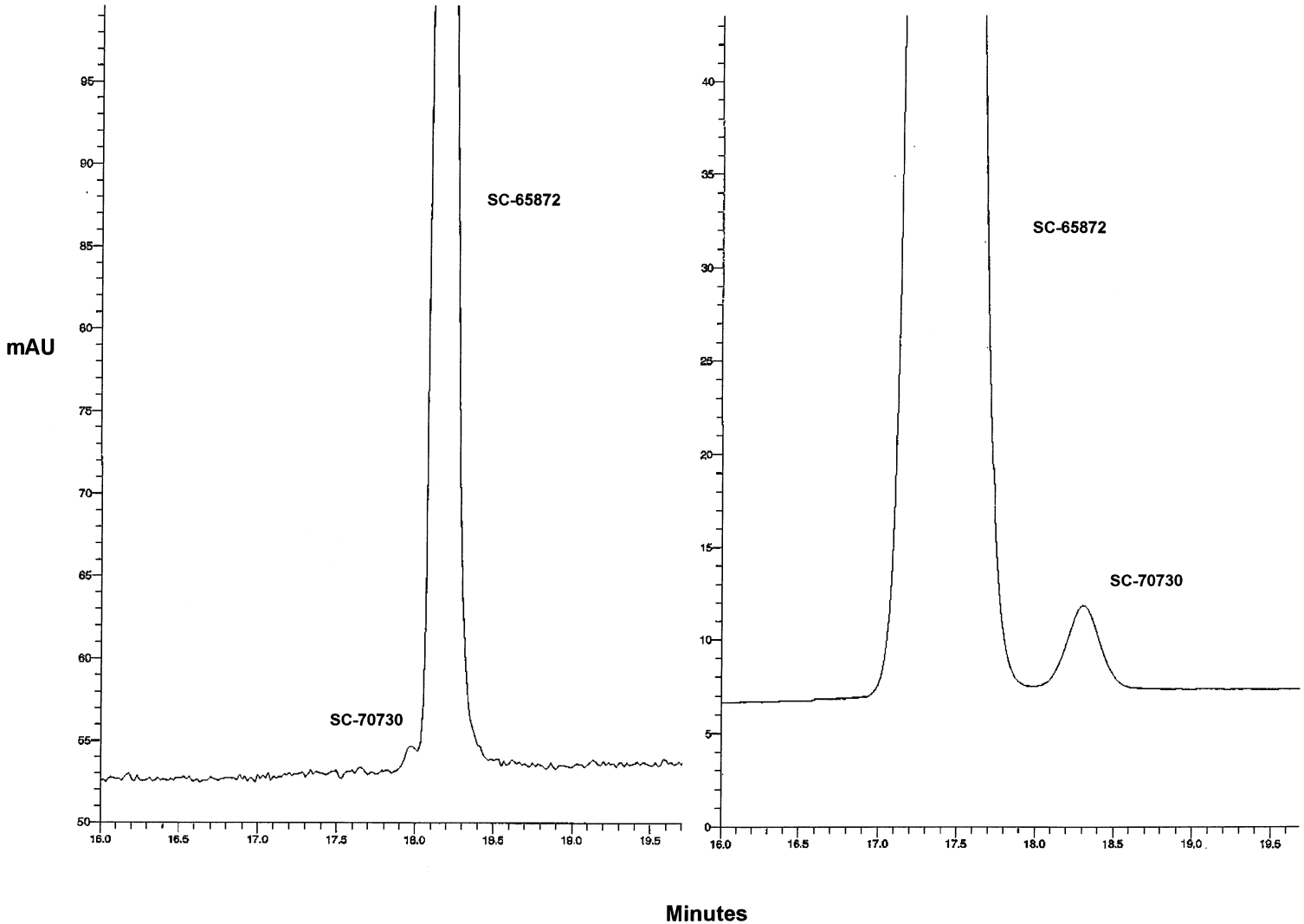
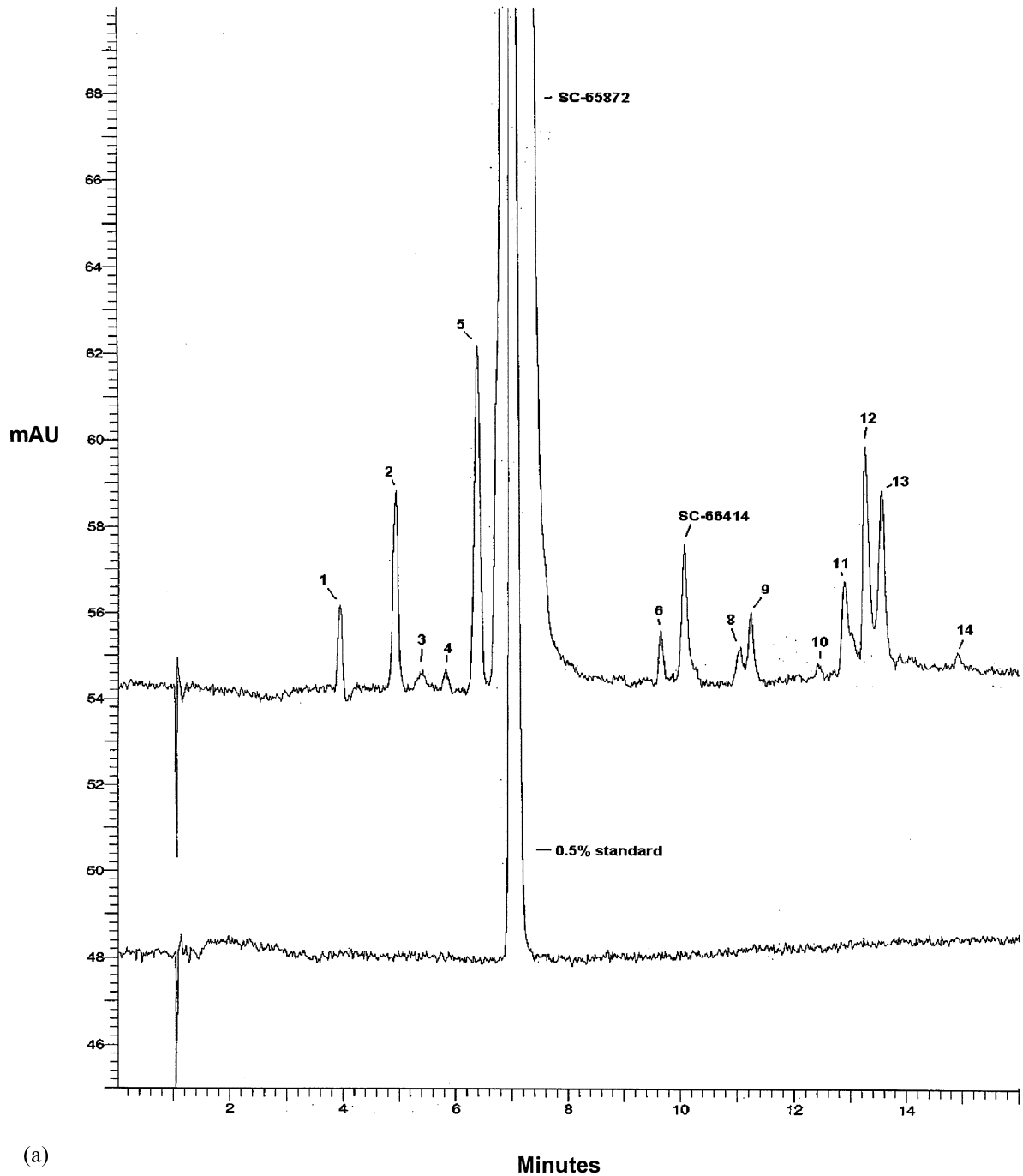


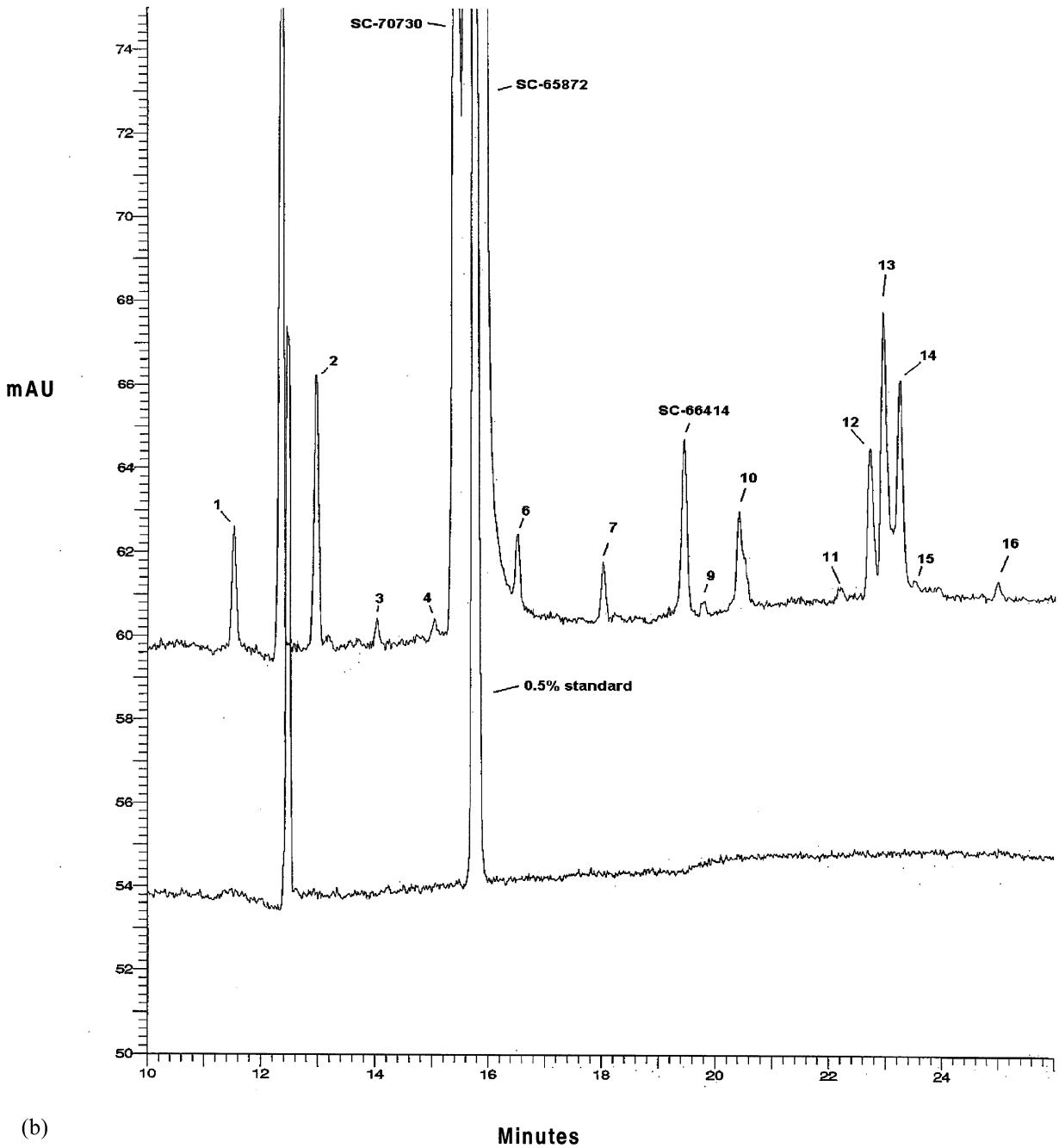
Fig. 4. PCSFC / HPLC sensitivity comparison for an 8 μg injection of SC-65872: A) PCSFC; B) HPLC.



(a)

Fig. 5. SC-65872 drug substance impurity profiles generated with PCSFC: (A) one column; (B) four columns; (C) six columns.

differences in the total impurity levels generated with PCSFC and HPLC could be attributed to differences in the absorptivities for SC-65872 and the known and unknown impurities at the detection wavelengths used with the procedures. RSD values ranged from 1.7 to 10.0% for the PCSFC



(b)

Fig. 5. (*Continued*)

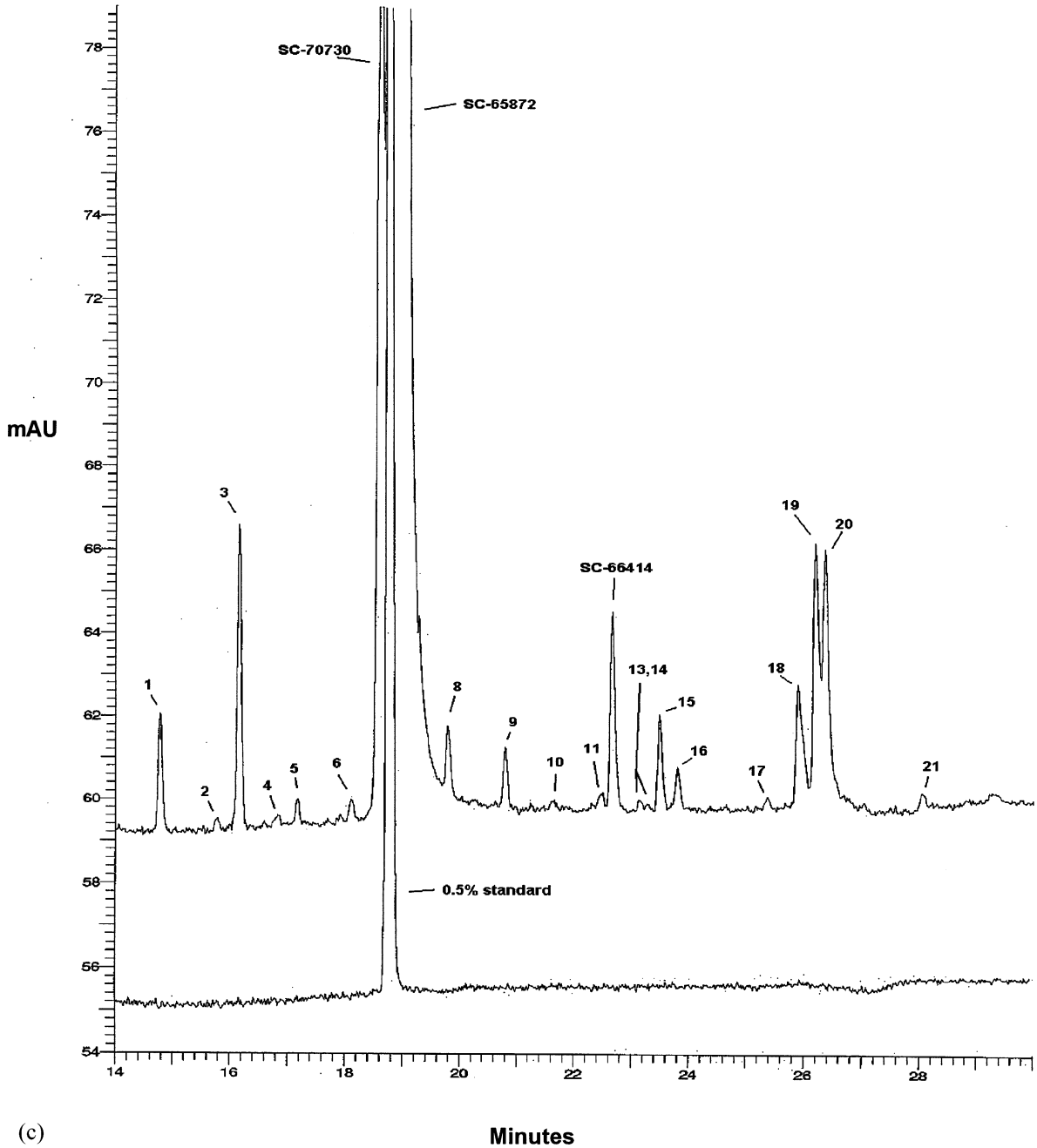


Fig. 5. (*Continued*)

determinations and 0.9 to 3.4% with HPLC. The superior precision generated with HPLC was due, in part, to the use of an autosampler with HPLC. The PCSFC results were generated with a manual injector. Precision was estimated for impurities quantitated at levels $\geq 0.01\%$ and based on tripli-

cate injections versus the SC-65872 external standard.

Even though extending column length during PCSFC increased the number of detected impurities, deleterious effects were observed for two sets of peaks as the column length was increased.

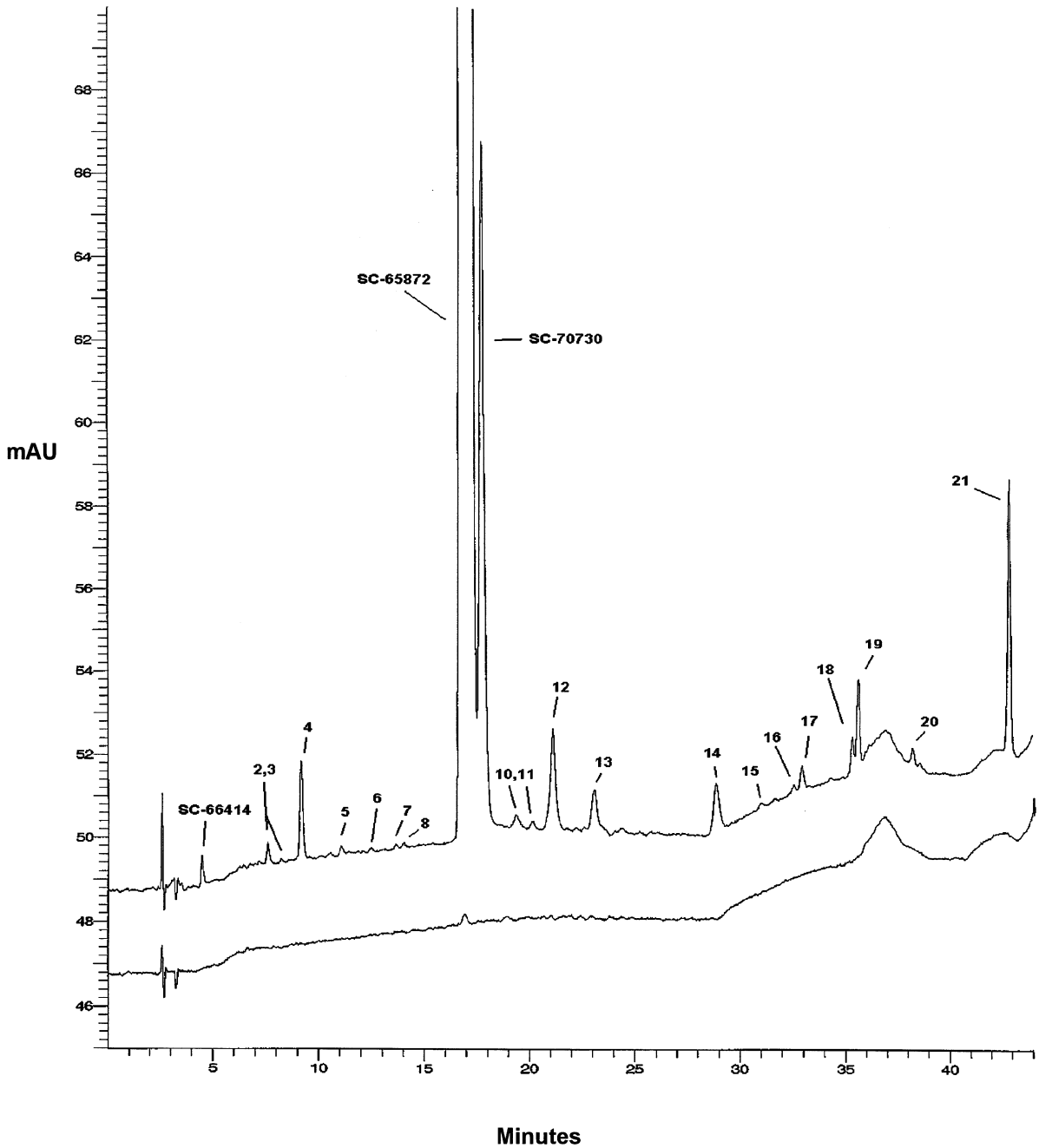


Fig. 6. SC-65872 drug substance impurity profile generated with HPLC.

Table 2
Quantitative Impurity Profiles Generated with PCSFC and HPLC Methods

PCSFC 1 column 125 μg			PCSFC 6 columns 125 μg			HPLC 1 column 16 μg		
Identity*	Level (%)	RSD	Identity*	Level (%)	RSD	Identity**	Level (%)	RSD
1	0.02	8.0	1	0.02	2.1	SC-66414	0.01	3.4
2	0.04	6.7	3	0.05	1.7	2	0.01	1.3
5	0.07	1.8	SC-70730	0.22	1.6	4	0.05	1.8
6	0.01	8.8	8	0.02	3.4	SC-70730	0.56	0.5
SC-66414	0.03	8.7	9	0.01	1.9	10	0.02	2.3
8	0.01	3.3	SC-66414	0.04	9.8	12	0.09	1.0
9	0.01	6.2	15	0.02	7.3	13	0.03	2.5
11	0.01	7.8	16	0.01	11.4	14	0.06	3.0
12	0.04	6.2	18	0.03	4.5	17	0.02	19.4
13	0.04	4.0	19	0.05	2.6	18	0.02	2.1
			20	0.06	10.0	19	0.04	1.0
						21	0.15	0.9
~4 impurities < 0.01%			~11 impurities < 0.01%			~9 impurities < 0.01%		
10 impurities > = 0.01%			11 impurities > = 0.01%			12 impurities > = 0.01%		
Total = 0.28%			Total = 0.53%			Total 1.06%		
Average RSD = 6.2			Average RSD = 5.0			Average RSD = 3.3		

* Refers to peak identities in Fig. 6A and 6C
** Refers to peak identities In Fig. 7

Peaks 8 and 9 were well-resolved during the one-column PCSFC experiment (Fig. 5A). When the column length is increased to four columns, the peaks appear to merge into a single peak (peak 10, Fig. 5B). As the column length increased to six columns in series, the peaks appear to be resolved with reversed elution order (note peaks 15 and 16, Fig. 5C). This effect would not be expected if the changes in the chromatograms were due solely to extension of the column length. One possible explanation is that the determinate factor for relative retention of peaks 8 and 9 in Fig. 5A was the increased pressure drop across the column as the length increased. During the PCSFC experiments, the initial inlet column pressure increased from approximately 150 bar to 290 bar as the number of columns increased from one to six. Since the outlet pressure was programmed at 120 bar for all PCSFC experiments, the pressure drop increased substantially as column length increased. The effect of column pressure drop during PCSFC and the impact on resultant chromatography has been the subject of considerable discussion ([19],[20]) with authors reporting various effects due to increased pressure drop. An additional deleterious effect was observed for the peaks 13 and 14 in Fig. 5B, which appear to have slightly decreased resolution as the column length is increased from four to six-columns (peaks 19 and 20 in Fig. 5C), due to band broadening.

4. Conclusions

Use of extended length columns during PCSFC is a useful concept in pharmaceutical industry laboratories where it is important to generate high-quality information with limited time and resources and where chromatographic profiles provide a fundamental assessment of the suitability of drug substance lots for use in clinical trials. Improving PCSFC separations by increasing column length affords the opportunity to assess the accuracy of profiles generated with single column procedures. For the drug substance sample analyzed in the current study, increasing column length revealed the presence of higher numbers of low level impurities. Also, the PCSFC/HPLC analyses completed in the present study generated comparable results, demonstrat-

ing that PCSFC can be used to verify the accuracy of results achieved with existing HPLC methods. An additional element of the impurity profile comparison is that most of the impurities detected with HPLC and PCSFC in the current study were 'unknown' impurities. Future studies will concern whether the HPLC and PCSFC are resolving the same or different impurities from the SC-65872 samples. If the procedures are revealing different 'sets' of impurities, the significance of tandem use of PCSFC and HPLC will be enhanced.

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