



Short communication

## Rapid analysis of polycyclic aromatic hydrocarbons

Justin M. Godinho\*, Jason Lawhorn, Barry E. Boyes

Advanced Materials Technology, Inc., 3521 Silverside Road, Wilmington, DE, 19810, USA



## ARTICLE INFO

## Article history:

Received 9 July 2020

Revised 22 July 2020

Accepted 24 July 2020

Available online 25 July 2020

## Keywords:

Polycyclic aromatic hydrocarbons

PAH

HPLC

Fast LC

Superficially porous particles

## ABSTRACT

Polycyclic aromatic hydrocarbons are a continuing environmental and health concern. The analytical methods developed to analyze this class of compounds have relied on reversed phase liquid chromatography and are often on the order of tens of minutes. Reduction in analysis times through the application of sub-2  $\mu\text{m}$  fully porous and superficially porous support materials can increase the throughput of these LC separations. Herein, we demonstrate similar selectivity between a fully porous 1.8  $\mu\text{m}$  and a 2.7  $\mu\text{m}$  superficially porous material. Separations were individually developed with *in silico* modeling for a given flow rate determined by the fully porous column's backpressure requirements. Since the 2.7  $\mu\text{m}$  superficially porous materials inherently require less backpressure to achieve similar levels of efficiency as the 1.8  $\mu\text{m}$  fully porous materials, a marked increase in throughput is possible with elevated flow rates. Good resolution for a standard 16-component sample mixture is demonstrated in a sub-minute separation.

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## 1. Introduction

Polycyclic (or polynuclear) aromatic hydrocarbons (PAHs) are a class of compounds composed of only carbon and hydrogen and contain high levels of aromaticity [1]. They are naturally occurring in traditional fuel sources and created during combustion processes of natural products [2,3]. Regular means of human exposure to mixtures of PAHs include breathing air contaminated with smoke and ingesting charred foods [4]. Effects of PAHs on human health are varied and have been shown to stretch from irritant to mutagen and carcinogen [5].

PAHs are widely identified in environmental samples and are an analytical challenge due to the complexity of the matrices they exist in and the plethora of isomeric forms [6]. This class of compounds continues to attract attention as environmental change and the depletion of conventional fossil fuels drive exploration of alternative fuel sources for heat and power [7].

Due to the analytical complexity of real world samples, analytical analysis workflows require the use of a sample preparation method, such as liquid-liquid extraction or solid phase extraction, followed by a chromatographic separation prior to detection [8]. Approaches, developed since the 1970's, utilize reversed phase liquid chromatography (RPLC) with UV absorbance or fluorescence detection or gas chromatography (GC) with mass spectrometry or flame ionization detection [8,9]. The Environmental Protection Agency has developed PAH-related methods for a range

of sample matrices that are often based on GC for its ability to afford greater selectivity, sensitivity and resolution in comparison to LC [10]. However, LC methods are still required for a unique separation of specific targets. For example, the GC procedure in Method 610 does not fully resolve four of the 16 target PAHs and requires a reversed phase LC separation [11].

Two of the most routinely employed advances for faster LC separations are sub-2  $\mu\text{m}$  fully porous particles (FPPs) and superficially porous particles (SPPs) [12]. Specific application of these advances to PAH separations has been reported. For example, different available column chemistries and particle sizes (from 5 to 1.8  $\mu\text{m}$ ) were compared for 7 PAHs with separation times as low as 2 minutes for the sub-2  $\mu\text{m}$  materials [13]. An example of core-shell technology was presented for a separation 8 PAHs in less than 15 minutes [14]. These reports suggest that this field has yet to fully realize the advantages of these high-throughput and ultrafast column technologies. Both materials, FPPs and SPPs, uniquely allow for an increase in column efficiency and reduction in experimental time. Small FPPs decrease chromatographic analysis time while maintaining efficiency and resolution at the expense of backpressure, and require high-pressure tolerances of all inline components of the LC system [15]. SPPs, alternatively, are attractive for high-throughput and ultrafast LC as they can often employ larger particles, requiring lower backpressures for optimal operation. Examples in the literature highlight similar efficiencies at 60% of the required pressure observed for sub-2  $\mu\text{m}$  FPP [16]. Moreover, SPPs maintain performance in higher velocity regimes than FPPs. In practice, these features are desirable as ultrafast separations are

\* Corresponding author.

E-mail address: [jgodinho@advanced-materials-tech.com](mailto:jgodinho@advanced-materials-tech.com) (J.M. Godinho).

typically conducted well in excess of the optimum column velocity, in order to reduce separation times [17].

Herein we demonstrate ultrafast and high throughput LC of a 16-component PAH mixture. We compare the *in silico* derived gradients for FPP and SPP columns and show similar selectivity and separation times. We further make use of the reduced backpressure requirements afforded by the SPP column to realize a fast separation of the PAH mixture in under 1 minute with a 1.5 minute method time, while operating at back pressures readily tolerated by commercial LC instruments.

## 2. Experimental conditions

### 2.1. Chemicals used

All solvents used were HPLC grade. Acetonitrile, uracil, individual standards and the analytical standard containing 16 PAHs in acetonitrile were obtained from MilliporeSigma (St. Louis, MO). The components of this PAH sample are part of the EPA 610 method [11].

### 2.2. Columns

Two HALO 90 Å PAH 2.7  $\mu\text{m}$  columns, a  $4.6 \times 150$  mm and  $4.6 \times 50$  mm, from Advanced Materials Technology, Inc. (Wilmington, DE) were used in these experiments (SPP column). This phase is a trifunctional, unendcapped, C18 on our 90 Å SPP support material. A  $4.6 \times 50$  mm ZORBAX Eclipse PAH column packed with 1.8  $\mu\text{m}$  95 Å FPP material with a PAH specific bonded phase (FPP column) was obtained for comparison (Agilent, Wilmington, DE). All columns were stored in acetonitrile between experiments.

### 2.3. HPLC equipment

LC experiments were conducted on a Shimadzu Nexera HPLC instrument (Columbia, MD). Chromatograms were detected using an UV diode array detector. The 254 nm wavelength is used for all depicted chromatograms. Data analysis was performed using the integrated LabSolutions software (Shimadzu).

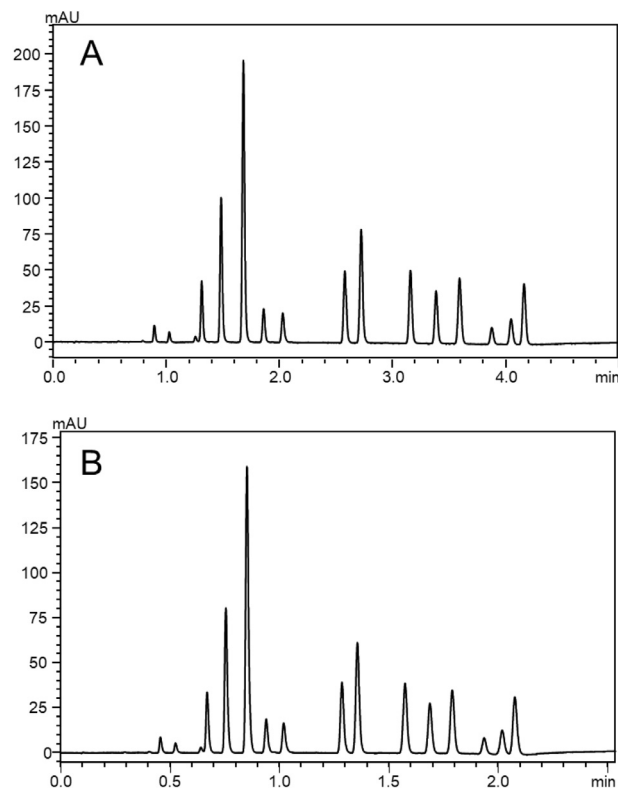
### 2.4. Dry lab

DryLab 2010 (Molnár-Institute, Germany) was used for the *in silico* derivation of separation conditions. Screening gradients probing the effects of temperature and gradient steepness were conducted to seed the analysis program. Four scouting gradients were run with the SPP  $4.6 \times 150$  mm column from 50 to 100% acetonitrile at 2.0 mL/min. These four gradients were comprised of 2, 10 minute, and 2, 20 minute runs, one at each operating temperature of 30°C and 50°C.

An additional set of modeling gradients for rapid analysis method development was conducted for the  $4.6 \times 50$  mm columns. These gradients were 50 to 100% acetonitrile in 10 and 20 minutes at 30 C and 2.0 mL/min. Results of all chromatograms were processed and manipulated in DryLab software with consideration given to balance between resolution and rapid analysis time. In all cases peaks were tracked using spectral matching from the UV diode array detector and peak areas.

## 3. Results and discussion

PAHs are well suited for traditional reversed phase separations as they do not contain complicating functional groups and exhibit consistent retention behaviors without mobile phase additives. Many phases have been developed and tested to improve and simplify the separation of PAHs and best results are often cited as



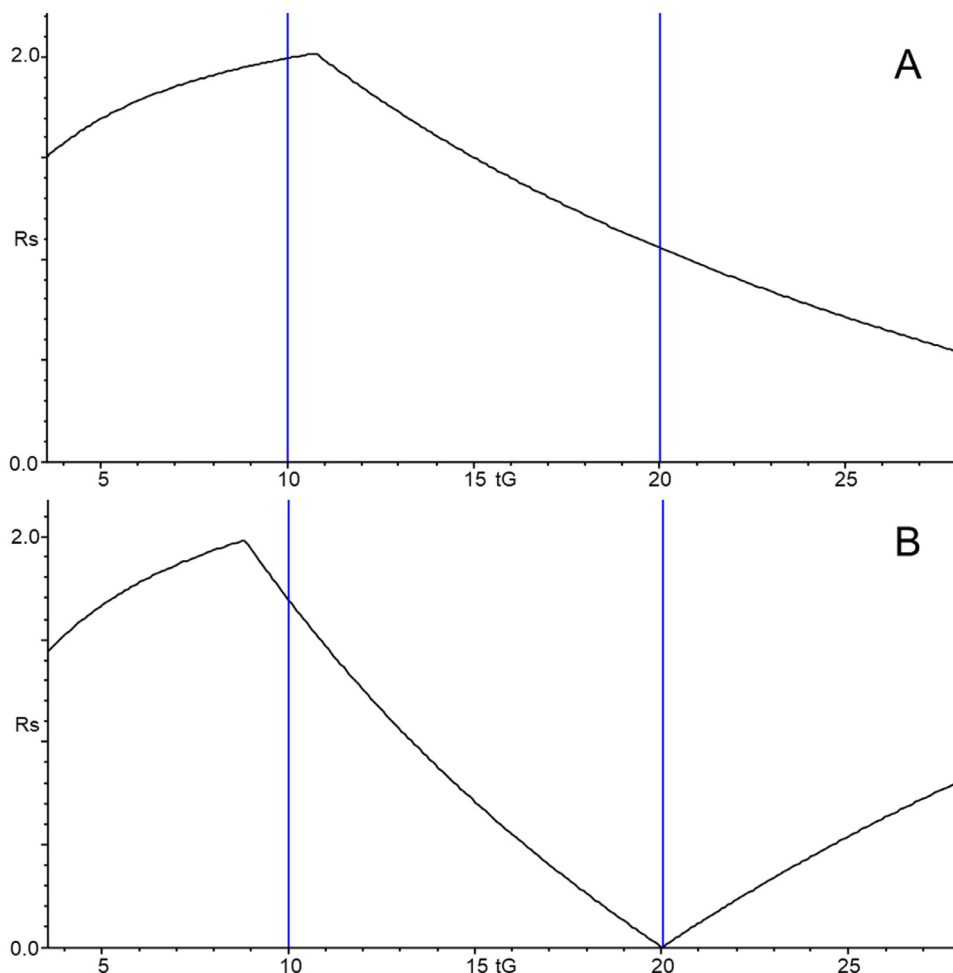
**Fig. 1.** Separation of 16 PAHs in 5 minutes (A) and 2.5 minutes (B) with a  $4.6 \times 50$  mm SPP column. The gradient for panel (A) was 50 to 100% acetonitrile in 4 minutes and held until 5 minutes at 2 mL/min. The separation in panel (B) was 50 to 100% acetonitrile in 2 minutes and held until 2.5 minutes at 4 mL/min.

a polymeric C18 [18,19]. To this end, we developed the HALO PAH phase on a 2.7  $\mu\text{m}$  SPP particle architecture with the ultimate goal of reproducible and rapid separations.

Our iterative development process was guided by DryLab software. This software allows us to streamline and facilitate the modeling, understanding and design of our experimental phases. We can rapidly screen and compare materials and conditions *in silico* after conducting limited and organized experimental gradients. The initial exploratory experiments were conducted with various prototypes to understand bonding and experimental variables affecting separation. Initial modeling experiments utilized 150 mm columns as this length eliminates plates as a restriction and highlights differences in selectivity that arise from prototype differences and experimental conditions. Importantly, the results of these modeling experiments indicated that adequate resolution could be obtained with 50 mm columns at 30°C.

Design of this phase was in part purposed towards the separation of a 16-component sample mix in 5 minutes with a minimum resolution of each pair of 1.4. As seen and described in Fig. 1A, a simple gradient, yields results exceeding our design criteria. Due to the gradient's simplicity, traditional methods for increasing the rapidity of a separation including doubling the flow rate and maintaining gradient steepness allow for a 2.5-minute separation. The results and gradient employed are shown in Fig. 1B. Here the lowest resolution value was for peak pair 3|4 at 1.40.

In addition to practical utility, as described above, the SPP architecture lends itself favorably to rapid separations. As a benchmark for our new material we chose a 1.8  $\mu\text{m}$  FPP material bonded with an application specific PAH phase. We ran matching experimental modeling gradients on the two  $4.6 \times 50$  mm columns. For direct comparison between the two materials we modeled gradients *in silico* for each column at an identical flow rate of 3 mL/min.



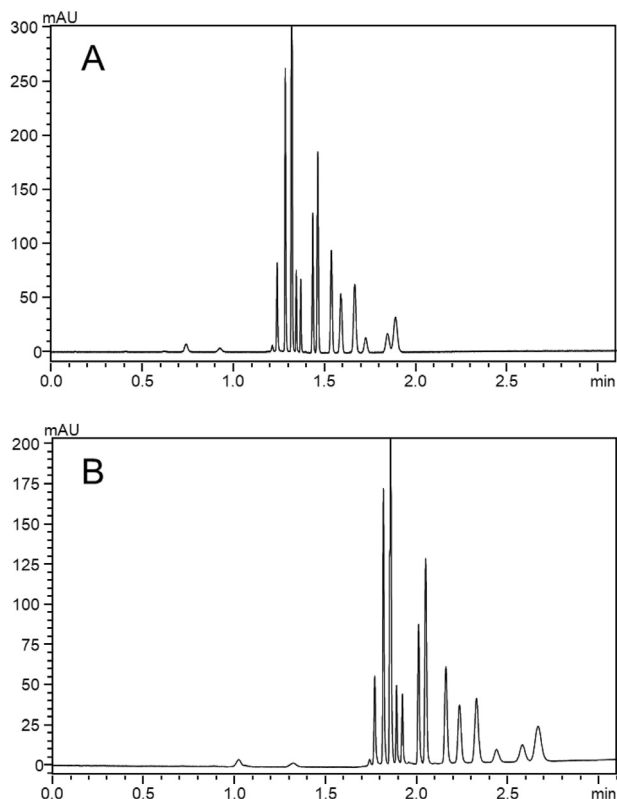
**Fig. 2.** Resolution plots as a function of gradient time for the SPP (A) and FPP (B) for  $4.6 \times 50$  mm columns at 3.0 mL/min at 30°C. The blue vertical lines indicate the initial experimental gradients used for modeling. Both columns exhibit similar resolution profiles. Critical pairs for the SPP on either side of the inflection point are 3|4 and 14|15 respectively. For the FPP critical pairs between inflection points are 3|4, 14|15 and 15|14. Notably, the resolution with the FPP material past 20 minutes begins to increase due to 14|15 critical pair inversion.

This flow rate was chosen as it produced a backpressure of 530 bar, within 10% of the recommended maximum column backpressure of 600 bar for the FPP column, in the initial mobile phase composition of 50/50 water/acetonitrile at 30°C. Under these conditions the SPP column's backpressure was 400 bar, a typical maximum backpressure for many standard pressure LCs. We noted that greater resolution of the critical 3|4 pair could be achieved with an initial isocratic hold at the beginning of the separation for both columns. Both modeled resolution vs time curves for the two  $4.6 \times 50$  mm columns are similar in terms of resolution for relatively short gradients under 10 minutes (Fig. 2). After 20 minutes, resolution for the FPP material begins to increase as this part of the separation condition topography appears dominated by an inversion of the 14|15 pair. The reason for this peak inversion is unknown but possibly due to a difference in the proprietary bonded chemistry. Practically, chromatographers often run as fast as possible, giving the comparative advantage to the SPP material for resolution and backpressure.

We used DryLab to model *in silico* gradient conditions that would meet a predicted minimum resolution of at least 1.5. These gradient profiles were run and the actual chromatograms are shown in Fig. 3. Notably both columns run under derived conditions have a resolution greater than 1.5 for each pair except for 15|16 (1.133 for the SPP and 1.356 for the FPP). Surprisingly, this resolution did not match modeling and could be due to the very

steep gradient slope and rapid switch from isocratic to gradient back to isocratic elution modes. Reports of the mass transfer effects of our 90 Å SPP support are well documented [20,21]. For this phase, we note an approximately 10% steeper C-term for naphthalene when compared to a traditional C18 bonded phase at a retention factor just greater than 3. We have found this slightly higher C-term to be specific to our PAH phase across column loading methods. Moreover, nearly identical minimum reduced plate heights of 1.5 confirm this C-term is directly related to the bonded phase. It is likely that some of our loss in predicted resolution is due to this design feature. That said, this iteration of bonded phase offers the most apt selectivity for the PAH compound class and furthermore, this selectivity impacts ultimate resolution more than partially restricted mass transfer.

The separation ordering for both the SPP and FPP columns is identical. In all chromatograms the elution order is 1) naphthalene 2) acenaphthylene, 3) acenaphthene 4) fluorene 5) phenanthrene 6) anthracene 7) fluoranthene 8) pyrene, 9) benzo(a)anthracene 10) chrysene 11) benzo(b)fluoranthene 12) benzo(k)fluoranthene 13) benzo(a)pyrene 14) dibenzo(a,h)anthracene 15) benzo(g,h,i)perylene 16) indeno(1,2,3-c,d)pyrene. The FPP column offered greater resolution for the 15|16 pair. This separation occurs during the 100% acetonitrile hold post-gradient. We believe separation of this pair is directly related to available total surface area of the support



**Fig. 3.** Experimental chromatograms, using conditions modeled *in silico*, depicting the SPP (A) and FPP (B) columns. These gradients are both at 3 mL/min flow rate. For the SPP column 50% acetonitrile was held for 1 minute and increased to 100% acetonitrile by 1.2 minutes. For the FPP column 50% acetonitrile was held for 1.5 minutes and increased to 100% by 1.7 minutes. Note the overall faster runtime for the SPP column.

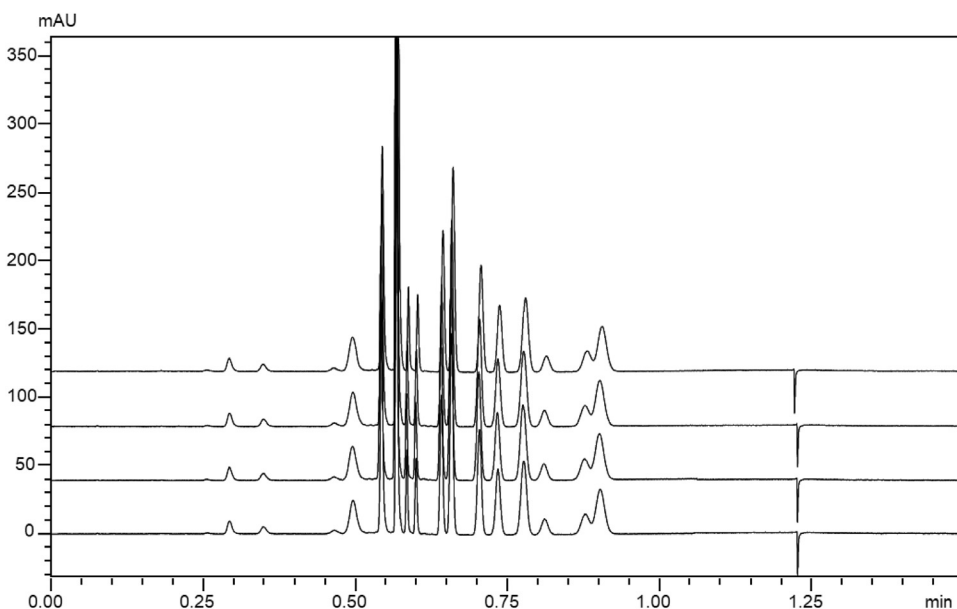
material, or alternatively the total amount of carbon on the material. The SPP column did successfully resolve this pair, with modestly lower resolution. Increasing resolution of this pair for both columns could be accomplished by reducing the limit of

100% acetonitrile, but with a concomitant expense of a longer total analysis time. The separation mechanism requiring the isocratic hold at the beginning of the run to increase the resolution of peaks 3|4 is unclear to us. However, the FPP required a longer hold than the SPP material and still did not reach the same level of resolution.

Given the lower backpressure for the SPP material we had an opportunity to increase the speed of the separation further and used DryLab to generate gradient conditions at 5 mL/min that would meet resolution of 1 in under 1 minute. At this flow rate the  $4.6 \times 50$  mm SPP column produced a backpressure of 630 bar. This pressure is well within specification for the Nexera LC (up to 1100 bar), and additionally gave us indication as to the stability and robustness of the column. Experimentally this gradient yielded a sub 1-minute separation with adequate resolution for qualification between each peak pair. Peaks 3|4 yielded a resolution of 1.790 and peak 15|16 had a resolution value of 1.08. This exemplifies our selection of bonded phase for PAH separations as plate height at this flow rate is relatively low, especially in comparison to the column's optimal linear velocity. The separation was replicated in order to test the ability of the column to regenerate between runs. A total method time of 1.5 minutes is shown in Fig. 4 and the gradient conditions are described in the caption. Reproducibility of these chromatograms is high indicating the rapid and sufficient equilibration required for high throughput analysis. Under similar conditions of operation, employing a 100 mm column at 600 bar back pressure, more than 500 replicate isocratic runs (14,000 column volumes of mobile phase), exhibited no changes in back pressure, compound retention, efficiency, or resolution.

#### 4. Conclusions

As energy sources continue to draw focused attention, accurate and efficient analysis of combustion byproducts will become ever more important. Here we demonstrate a high throughput method and reversed phase material for the analysis of PAHs, which can readily enter the biosphere, with potential environmental and physiological consequences. We directly compare two column supports with a similar bonded phase and show that both can significantly reduce total analysis time. SPP materials have an



**Fig. 4.** Replicate injections of the less than 1 minute PAH separation with a 1.5 minute total method time. In this gradient, 58% acetonitrile was held until 0.4 minute, increased to 100% acetonitrile at 0.5 minute, held until 1.1 minutes, decreased back to 58% acetonitrile at 1.11 minutes. Method stop was at 1.5 minutes.

inherent advantage in terms of increased efficiency at very high linear velocities and reduced backpressure requirements compared to the sub-2  $\mu\text{m}$  FPP materials. Taking advantage of the larger particle size, reduced backpressure and highly-efficient SPP morphology allows for the development of a robust, rapid and well resolved PAH separation in less than 1 minute with a 1.5 minutes total method time. **To our knowledge this is likely one of the fastest separation examples of this class of compounds using a reversed phase LC method.** Future developments will likely include a significant reduction in solvent consumption and increased sensitivity through the use of smaller bore columns or alternatively completely different hardware and modes of separation such as in a recent report on turbulent supercritical fluid chromatography in open-tubular columns where a selection of 4 PAHs were resolved in under 9 seconds [22].

### Declaration of Competing Interest

The authors are employed by a company that manufactures some of the materials referenced in this article.

### CRediT authorship contribution statement

**Justin M. Godinho:** Conceptualization, Methodology, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Jason Lawhorn:** Resources, Methodology. **Barry E. Boyes:** Conceptualization, Methodology, Writing - review & editing, Supervision.

### Acknowledgments

The Authors would like to thank Tom Waeghe (MAC-MOD Analytical, Chadds Ford, PA) for his help with DryLab software. The authors would also like to thank Joseph DeStefano, Tiffany Jones and Conner McHale (Advanced Materials Technology, Inc., Wilmington, DE) for helpful discussions, column production and individual standard identification respectively.

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