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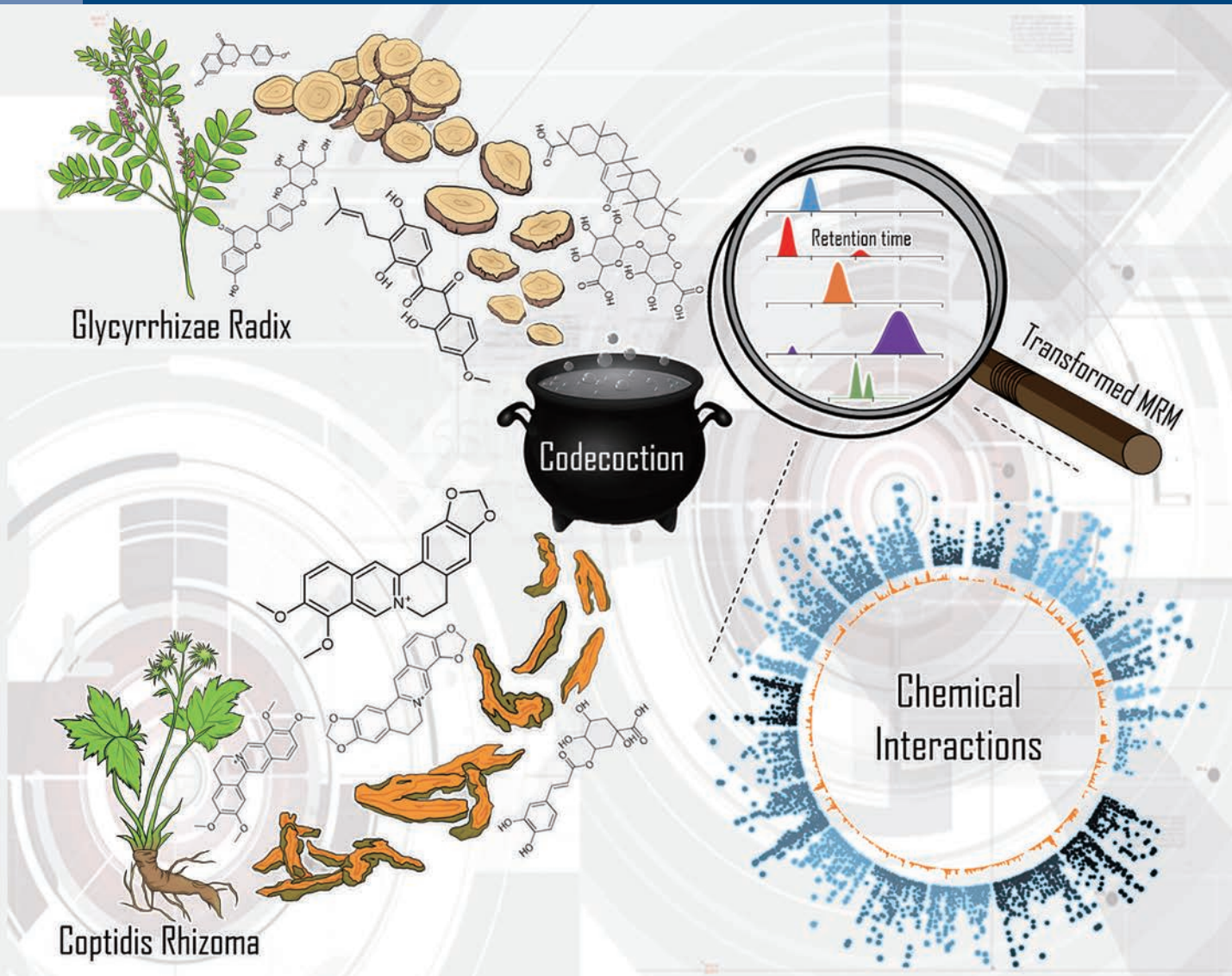

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RESEARCH ARTICLE

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Multilayered particle-packed column: Evaluation and comparison with monolithic and core–shell particle columns for the determination of red azo dyes in Sequential Injection Chromatography

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A recently presented new type of "multilayered" organic–inorganic hybrid silica particle packed column YMC-Triart C_{18} (50 mm × 4.6 mm, 5 µm) was used for the development of a sequential injection chromatography method for determination of five azo dyes (Sudan I, Sudan II, Sudan III, Sudan orange G, and para red) in selected food seasonings. The use of a novel sorbent brings attractive features, reduced backpressure, and broader chemical stability together with high separation performance, which are discussed and compared with that of three types of columns typically used in medium-pressure flow chromatography techniques (classic monolithic, narrow monolithic, and core–shell particle columns). The separation was performed in gradient elution mode created by the zone mixing of two mobile phases (acetonitrile/water 90:10, 1.5 mL + acetonitrile/water 100:0, 2.3 mL) at a flow rate of 0.60 mL/min and time of analysis <9.5 min. The spectrophotometric detection wavelengths were set to 400, 480, and 500 nm. The high performance of the developed method with multilayered particle column was well documented and the results indicate a broad capability of sequential injection chromatography.

KEYWORDS
azo dyes, columns comparison, multilayered particle column, sequential injection chromatography

1 | INTRODUCTION

Sequential injection chromatography (SIC) is based on a highly versatile sequential injection analysis manifold (SIA) characterized by a bidirectional syringe pump and a multiposition selection valve that enable programmable and controllable flow of the liquids and controlled zones dispersion in tubes of submillimeter internal diameter. The technique was introduced by Ruzicka in 1990 [1] and the fundamentals of zones dispersion in the typical SIA flow design were described in detail by Gübeli in 1991 [2]. SIC young and quickly developing technique has earned a place among the LC analytical techniques since 2003 when introduced by Šatínský [3]. Within 13 years of gradual development, our group has presented several important steps in the development of the SIC technique. Some of them are the use of different monolithic columns with lengths 10, 25, 50, and 100 mm, and with a classic 4.6 mm or with a narrow-bore 2 and 3 mm inner diameters in a single column [4–6], or a parallel columns configuration [7]. Later, the SIC development continued using core–shell 2.7 µm particle-packed columns [8]. Several workgroups worldwide contributed to the development of the SIC by new approaches and methods

Abbreviations: SIA, sequential injection analysis; SIC, sequential injection chromatography

Conflict of interest: The authors have declared no conflict of interest.

too. Significant works are a method for parabens separation developed by Batista with a separation method for micellar mediated for determination of melamine in milk [9]; a method for measurement of oxalate in beer and urine samples using a surfactant-coated C_{18} monolithic column developed by Maya [10]; a method for separation of three water soluble textile azo dyes using multiisocratic mode with two different ion pair reagents in mobile phase developed by Fernández [11] and a method for separation of proteins using cation exchange polymeric monolithic column developed by Masini [12]. The medium pressure flow system based on the SIC are still represented by low system dead volume, low gradient profile delay, low mobile phase consumption, and overall simple operation.

Monolithic sorbents or 2.7 μm core–shell particle packed columns have been typically used in the SIC. They create acceptable low resistance to mobile phase (backpressure) therefore are compatible with the SIC manifold with a system pressure limit of 750 PSI (5.17 MPa) [8]. Evident advantage of the particle packed columns over commercially available monolithic columns is broad choice of producers, column dimensions, sorbent particle sizes, and chemistries (reversed phases: C_{18}, C_8, Amide, Pentafluoropropyl, Phenyl-Hexyl, Cyano normal phase, HILIC, ion exchanger, etc.) bringing variable separation selectivity. This facilitates the development of separation method by choice of optimal stationary phase according to the kind of separated analytes and a sample matrix. The importance of selection of an appropriate chromatographic column with respect to analytes and analyzed samples in the SIC was proven by the development of on-line hyphenated SPE and LC method using SIC manifold as an example of advanced automated analytical technique [13].

The multilayered organic/inorganic hybrid silica material of particles used in this work follows trends in fast LC. The technology brings new features compared to commonly used fully porous and superficially porous particles (fused-core or core–shell). The advantages are working pH range 1–12, high physical-chemical stability, narrow particle, and pore size distribution that brings reduced system backpressure (30% lower than porous particles). The separation efficiency of the multilayered particle columns is expected to be comparable to the core–shell particle columns of similar particle size. To evaluate the multilayered column, together with development of a new SIC method, three types of column sorbents will be compared in terms of properties important in LC (backpressure, peak symmetry, separation performance, column dead volume, available sorbent chemistries, column physical-chemical stability, column price, and analysis costs). Backpressure is the most limiting property for SIC since it imposes limit to the flow rate and to the sample throughput. Total column score could suggest the reliability of the column for the SIC.

Organic azo dyes of Sudan family are highly stable orange-red industrial colorants (coloring oils, plastics, waxes, etc.) but with illegal food and beverage use (according to the European Food Safety Authority) since they are classified as genotoxic and/or carcinogenic [14]. However, these dyes are still abused in several countries to intensify red color making food products attractive. Many articles describing separation and determination of azo dyes has been published, dominantly using RP LC since reliable separation method can help to reveal the presence of illegal/fake substances in food and beverages [15–17]. The SIC method was developed for determination of five selected azo dyes possibly presented in chili-based spice mixtures and seasonings. Selected azo dyes were Sudan I, Sudan II, Sudan III, Sudan Orange G, and Para Red (chemical structures depicted in Supporting Information Fig. S1).

This work describes the extension of chromatographic abilities of the SIC using new type of a particle packed column with critical comparison of the results with three typically used kinds of C_{18} columns (classic monolith, narrow monolith, and core–shell particle packed). Standard solutions of selected azo dyes were used to test the columns and to validate developed method; however, samples of three seasonings spiked with 2.50 mg/L of all selected azo dyes were analyzed using liquid extraction with tetrahydrofuran as a sample pretreatment.

2 | MATERIALS AND METHODS

2.1 | Apparatus

The chromatographic method was performed by commercially available instrument based on SIChrom™ (FIAlab® Instruments, Bellevue, WA, USA) equipped with a 4.0 mL S17 PDP syringe pump (Sapphire™ Engineering, MA, USA) with a holding coil (PEEK, 1.75 mL, 396 cm × 0.75 mm id) on upper front port and an eight-port high-pressure stainless-steel selection C5H valve (VICI®, Valco Instrument, Houston, TX, USA). Flow lines were made of 0.25 mm (connection between the selection valve and a column inlet, and a column outlet and a detector flow cell), 0.50 mm (inlet for sample) and 0.75 mm id (holding coil and all other lines) PEEK tubing (VICI®, Valco Instrument, Houston, TX, USA). Spectrophotometric detection in the visible range was performed with a USB 4000 fiber optics CCD UV–VIS detector (Ocean Optics, Dunedin, FL, USA), a tungsten light source HL-2000 (Ocean Optics, Dunedin, FL, USA) and a SMA connector Ultem® micro-volume (9 μL) Z-flow cell of the detector with an optical path of 20 mm (FIAlab® Instruments, Bellevue, WA, USA). The SMA connector ended fiber optic cables with a core diameter of 600 μm (CeramOptec®, East Longmeadow, MA, USA) were used for light transmission (lamp-flow cell and flow cell-detector). The upper rear pump port was connected with an Alltech

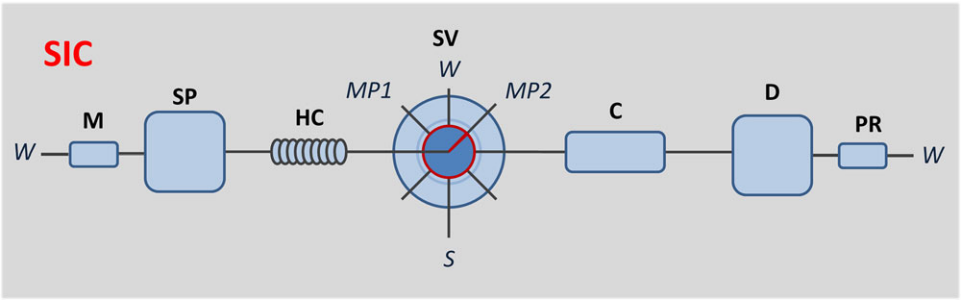

FIGURE 1 Scheme of SIChrom™ manifold: C–chromatographic column, D–vis detector, HC–holding coil, M–manometer with pressure release 750 PSI, PR–pressure release 20 PSI, SP–syringe pump, SV–selection valve; MP1–mobile phase 1, MP2–mobile phase 2, S–sample, W–waste

AP19258 1/16"online manometer with 0–3000 PSI gauge (Alltech, Czech Republic) and with a system pressure safety 750 PSI relief valve enabling real-time monitoring of the system pressure and pressure protection of the system. The 20 PSI relief valve at the outlet of the detector flow cell prevented the spontaneous flow of the mobile phase in the lines when the pumping was stopped. The SIC system was controlled and data from detector collected by a PC equipped with FIAlab® 5.9 software (FIAlab® Instruments, Bellevue, WA, USA). The SIC manifold setup is depicted in Fig. 1.

Within the separation method development were tested and compared four different chromatographic columns with C_{18} functional groups: multilayered organic/inorganic hybrid silica particle column YMC-Triart C_{18} (50 mm × 4.6 mm, 5 µm) (YMC, Japan); classic monolithic column Chromolith® SpeedROD RP-18e (50 mm × 4.6 mm) (Merck, Germany); narrow monolithic column Chromolith® FastGradient RP-18e (50 mm × 2 mm) (Merck, Germany) and core–shell particle packed column Ascentis® Express C_{18} (30 mm × 4.6 mm, 2.7 µm) (Supelco, USA).

2.2 | Chemicals

Sudan I (SI, 1-Phenylazo-2-naphthol, 97%), Sudan II (SII, 1-(2,4-Xylylazo)-2-naphthol, 90%), Sudan III (SIII, 1-[4-(phenylazo)phenylazo]-2-naphthol, 90%), Sudan Orange G (SOG, 2,4-dihydroxyazobenzene, ≥97%), Para Red (PR, 1-(*p*-nitrophenylazo)-2-naphthol, 95%), tetrahydrofuran anhydrous, ≥99.9%, methanol and acetonitrile CHROMASOLV® gradient grade, for HPLC, ≥99.9% were obtained from Sigma–Aldrich® (Czech Republic). Millipore Milli-Q RG ultrapure water (Millipore™, Czech Republic) was used throughout the experiments.

2.3 | Standard solutions and mobile phases

Standard stock solutions were prepared by dissolving azo dye standards in tetrahydrofuran at a concentration of 1000 mg/L and were stored at 5°C for 1 month. Tested mixtures of working standard solutions with the dyes were diluted with the mobile phase 1 before the analysis to demanded concentration. These solutions were used for SIC method development and validation, and for column characterization and evaluation of the chromatographic parameters. The final gradient conditions were set by mixing of the mobile phase 1 (acetonitrile/water 90:10, 1.5 mL and the mobile phase 2 (acetonitrile/water 100:0, 2.3 mL). The mobile phases were degassed before use by sonication for 10 min.

2.4 | Samples and sample handling

The tested samples were selected seasonings (chili products): "ABC Chili Sauce Hot" (liquid) and "Feferóny Mleté v 20% soli" (dry spice mixture) and "El Carmen – Mojo Típico Palmero" (chili powder) purchased in a supermarket in Czech Republic. Sample pretreatment was done by following procedure: 1.0 g of each sample was weighed in plastic 50 mL centrifuge tube with screw cap and mixed with 10 mL of tetrahydrofuran. Spiked samples were fortified with the stock standard solution of the five azo dyes to final concentration of 5.00 mg/L each respective dye. The samples were thoroughly mixed and stored 24 h at 5°C, then they were sonicated for 15 min in ultrasonic bath. The collected supernatant was filtered (0.45 µm pore sized filter, Polytertrafluorethylene) and diluted twice with 50% acetonitrile in water, so the final concentration was 2.50 mg/L of each dye. There were prepared three samples of each kind of chili product. The comparative standard solutions were of the same concentration. They were prepared similar way to the samples by diluting the stock standard solution with tetrahydrofuran and 50% acetonitrile to final concentration of the analytes of 2.50 mg/L. Standards and samples were measured in duplicate and the mean peak height values were used for recovery calculation. The concentrations of azo dyes in the samples were not determined since none of tested samples contained detectable amount of

analyzed dyes, so the samples tested mainly the level of matrix interferences.

2.5 | System validation

The chromatograms were processed and the peak heights were used for quantification (FIAlab® 5.9. software does not allow for easy and precise calculation of the peak area). Basic chromatographic parameters were calculated–a retention time, a peak symmetry, a resolution, and a peak capacity following the Ph. Eur. [18] and work of Jandera characterizing gradient elution conditions [19].

The selected validation parameters (calibration range, retention time repeatability, precision, and limits of detection and quantification) yielded results comparable with other SIC methods. The calibration ranges of azo dyes were established with a series of working solutions prepared by diluting the stock standard solutions with the mobile phase 1 to the final concentrations. Six working standard solutions of increasing concentrations (0.50–30.00 mg/L) were used. The LODs and LOQs were set as concentrations at which the working standard solutions gave the analyte signal to baseline noise ratios equal to 3 and 10, respectively. The system precision and repeatability of the retention times, expressed as RSD, were determined from five repeated injections of the working standard solution (10 mg/L). Recoveries of all analytes were determined in three samples spiked with standard solutions at 2.50 mg/L level.

3 | RESULTS

The SIC method was developed following the long-time experience of authors with flow methods and results of Khalikova using an on-line SPE–UHPLC–UV/vis method employing fused core particle columns for extraction, separation, and quantitative analysis of nine dyes illegally present in food [20]. The development was focused on the selection of the separation conditions that provide the highest separation efficiency as well as a simple, fast, and cost-effective analysis. The applicability of the developed method was tested on spiked samples of selected food seasonings pretreated with liquid extraction with tetrahydrofuran.

3.1 | Method development

The first tests of the new multilayered particle column in the SIC were carefully done focusing on a system backpressure value, a system leak tightness, applicable mobile phase flow rates and a mobile phase composition. The optimization of a mobile phase composition was initiated by selecting a suitable organic modifier, methanol, or acetonitrile. Methanol-based mobile phases were tested with less than satisfactory results and higher backpressure, while the acetonitrile-based mobile phases showed higher separation efficiency and lower backpressure. Then, the optimization of mobile phase was focused on selection of an appropriate acetonitrile/water ratio to achieve optimal separation of all analyzed dyes within a short analysis time (using the highest possible flow rate with a reasonable working pressure) and a single fill-up of the pump reservoir (4.0 mL) for whole separation. Separation flow rate was set to 0.60 mL/min and total volume of mobile phase was 3.8 mL. The pH of the mobile phase was not adjusted since the lipophilic analytes are not ionized within neutral pH. None of the selected conditions under the isocratic elution mode separated all dyes, so the gradient elution mode using two mobile phases was used. The gradient elution was developed by mixing of mobile phase 1 and mobile phase 2 in the holding coil of the pump testing different concentration of acetonitrile and volume of particular mobile phase. The process of mixing was: mobile phase 2 was aspirated from the valve port 2 through the holding coil into the reservoir of the pump and subsequently mobile phase 1 was aspirated from the valve port 8 into the holding coil (mutual mixing was boosted by a coiled tube), then the valve was switched to the valve port 3 (column) and flow was reversed for the sample separation. The volume of mobile phase 1 was always lower than 1.5 mL to hold gradient and mobile phase 1 zones in the holding coil (1.75 mL), and to prevent their aspiration inside the reservoir of the pump. The final conditions were: the mobile phase 1–acetonitrile/water 90:10, 1.5 mL and the mobile phase 2–acetonitrile/water 100:0, 2.3 mL. Total mobile phase volume of 3.8 mL (single aspiration of the syringe pump) was used to elute all of the separated analytes within one separation cycle. The column was conditioned at the end of measurement with 1.0 mL of mobile phase 1. The working pressure within the separation was 300–400 PSI (2.06–2.76 MPa) under final conditions (flow rate 0.60 mL/min), thus sufficiently lower than the system-pressure limit of 750 PSI (5.17 MPa). This ensure stable results of the real sample analysis. Injected volume of the analyzed sample was 10.0 μL concerning the recommended and tested capacity of the column and precision of the pump. The detection wavelengths 400, 480, and 500 nm were chosen based on the visible spectra of the analyzed compounds. Selected wavelengths provided sufficient measurement sensitivity for the analytes and easy peak identification during the method development. The detector was set to scanning rate of 7 Hz and integration time of 80 ms. The developed method using the multilayered particle column allowed for separation of five target analytes in less than 9.5 min (0.95 min for both mobile phases aspiration, 0.02 min for sample aspiration, 6.36 min for separation and 1.95 min for column conditioning). The sequence of the specific steps used in the SIC control program for the separation of all of the

TABLE 1 The sequence of particular steps of the program for gradient elution SIC method

Action	Unit	Parameter
Mobile phase 2 aspiration	Selection valve	Valve port 2
	Pump	Flow rate 4.2 mL/min
	Pump	Volume 2.30 mL
Mobile phase 1 aspiration	Selection valve	Valve port 8
	Pump	Flow rate 4.2 mL/min
	Pump	Volume 1.50 mL
Sample aspiration	Selection valve	Valve port 4
	Pump	Flow rate 0.6 mL/min
	Pump	Volume 0.01 mL
Gradient elution	Selection valve	Valve port 3
	Pump	Flow rate 0.6 mL/min
	Pump	Volume 3.81 mL
Mobile phase 1 aspiration	Selection valve	Valve port 8
	Pump	Flow rate 4.2 mL/min
	Pump	Volume 1.00 mL
Column conditioning	Selection valve	Valve port 3
	Pump	Flow rate 0.6 mL/min
	Pump	Volume 1.00 mL

substances (a single cycle) is described in Table 1. The measurements were performed in triplicate at an ambient temperature (25°C).

3.2 | Chromatographic characteristics and figures of merit

The separation of five azo dyes was carried out under developed conditions using multilayered and all other tested columns. The data characterizing the separation and the analytical parameters of separation are summarized in Table 2. The SIC chromatogram demonstrating the separation of the standard solution of five azo dyes is depicted in Fig. 2A.

Basic chromatographic parameters proved the performance of the method based on the multilayered particle column and were used for critical comparison with other selected columns. However, several parameters were worse in comparison with method using core–shell particle column (peak symmetry, resolution, and peak capacity), still they were better than with methods using classic monolithic and narrow monolithic columns.

The method parameters showed the calibration range 0.50–20.00 mg/L (only SOG range was 1.00–30.00 mg/L). The limits of detection and quantification were the same: LODs 0.15 mg/L and LOQs 0.50 mg/L for all the analytes except for SOG (LOD = 0.30 mg/L and LOQ = 1.00 mg/L). System precision described by RSD was ≤ 2.16% for peak quantification and ≤ 0.16% for retention time (10 mg/L, $n = 5$). The details are summarized in Table 2.

TABLE 2 Validation results and analytical parameters of SIC separation of five azo dyes using multilayered particle column

	SOG	PR	SI	SII	SIII
Calibration range (mg/L)	1.00–30.00	0.50–20.00	0.50–20.00	0.50–20.00	0.50–20.00
Correlation coefficient	0.998	0.999	0.999	0.999	0.997
Slope	$2.808 \times 10^{-2} \pm 8.60 \times 10^{-4}$	$4.420 \times 10^{-2} \pm 4.00 \times 10^{-4}$	$2.48 \times 10^{-2} \pm 1.90 \times 10^{-4}$	$1.64 \times 10^{-2} \pm 1.60 \times 10^{-4}$	$2.26 \times 10^{-2} \pm 8.40 \times 10^{-4}$
Intercept	$6.87 \times 10^{-3} \pm 1.32 \times 10^{-2}$	$7.27 \times 10^{-3} \pm 3.80 \times 10^{-3}$	$6.39 \times 10^{-3} \pm 1.80 \times 10^{-3}$	$1.86 \times 10^{-3} \pm 1.50 \times 10^{-3}$	$-1.59 \times 10^{-2} \pm 7.90 \times 10^{-3}$
Limit of detection (mg/L)	0.30	0.15	0.15	0.15	0.15
Limit of quantification (mg/L)	1.00	0.50	0.50	0.50	0.50
System precision (RSD%, $n = 5$) 10 mg/L	1.51	1.63	1.08	0.83	2.16
Repeatability of T_R (RSD %, $n = 5$) 10 mg/L	0.16	0.09	0.09	0.06	0.09
Retention time (min)	1.55	2.47	3.19	4.28	5.06
Peak symmetry	1.50	1.33	1.23	1.20	1.46
Peak resolution	5.71	3.75	6.06	4.78	
Peak capacity	17.6	13.1	12.0	15.7	14.3

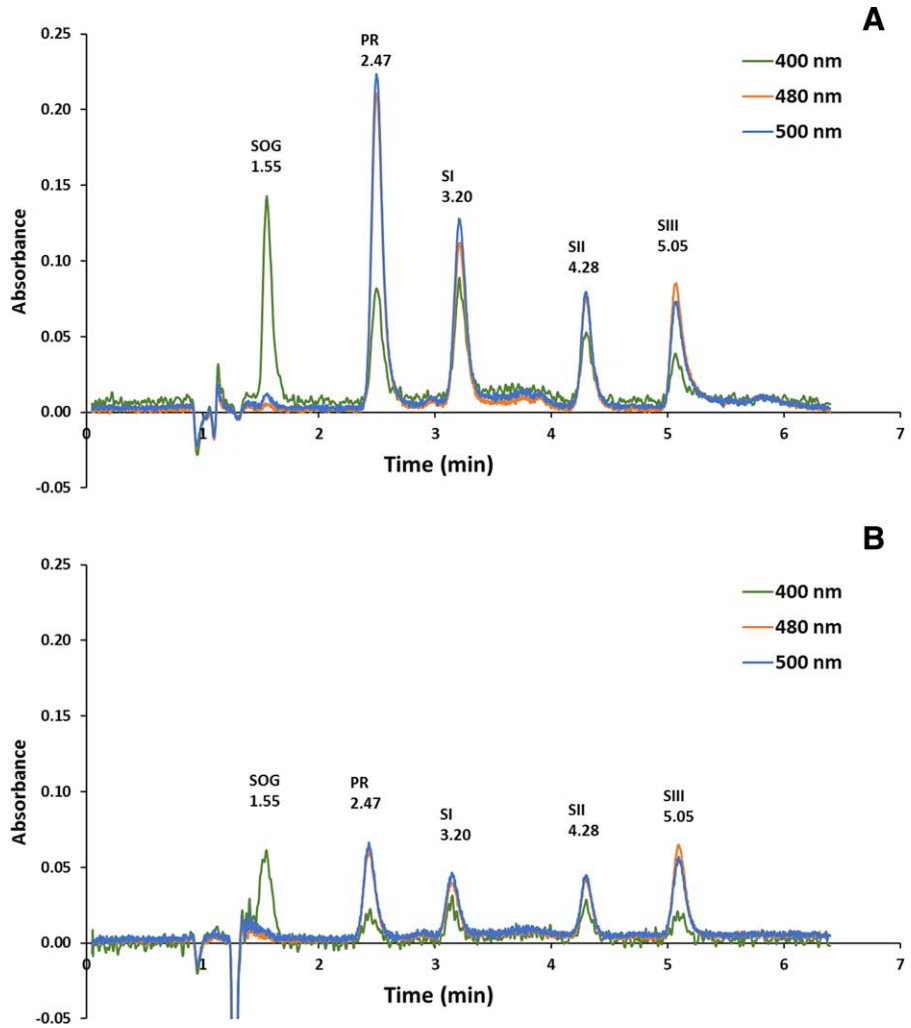

FIGURE 2 Chromatograms of (A) mixture standard solution (5.00 mg/L each standard), (B) sample ABC Chili Sauce Hot (spiked 2.50 mg/L each standard, after sample pretreatment), YMC-Triart C_{18} (50 mm × 4.6 mm, 5 µm), gradient elution (acetonitrile/water 90:10, 1.5 mL + acetonitrile/water 100:0, 2.3 mL), flow rate 0.60 mL/min, injection volume 10 µL

3.3 | Sample analysis results

The method LODs and LOQs for selected analytes in standard solution met the requirements of the European Regulation that established the detection limit for Sudan colorants in food matrices in range of 0.5–1.0 mg/kg [14]. Three selected samples of food seasonings did not contain detectable amount of forbidden azo dyes (measured by online SPE–UHPLC–UV/vis method [20]) and any interfering peaks of matrix of other substances were present in the chromatographic records. Spiked samples were used for testing the method applicability. The samples, three different matrices were spiked with selected dyes, used for recovery measurements, and the values were 84.3–89.1% (n = 3) for the liquid matrix, 94.3–97.2% (n = 3) for the dry spice mixture and 101.9–108.5% (n = 3) for the chili powder. The results proved the accuracy of the developed method for determination of selected azo dyes in three typical kinds of chili based products. The detailed results are given in Supporting Information Table S1. The representative SIC chromatogram demonstrating the separation of the sample spiked with five azo dyes (2.50 mg/L each standard) is depicted in Fig. 2B.

4 | DISCUSSION

4.1 | Separation method

High separation efficiency was reached with all tested columns although the flow rate used (0.6 mL/min) was lower than the optimum flow rate determined by Van Deemter plot. The peak capacity values confirmed a performance of

TABLE 3 Comparison of separation parameters of four types of columns used for separation of five azo dyes in SIC under the same conditions–gradient elution (acetonitrile/water 90:10, 1.5 mL + acetonitrile/water 100:0, 2.3 mL), flow rate of 0.60 mL/min, mixture standard solution (each 10 mg/L), sample injection 10 µL

Column name	YMC-Triart C18	Merck Chromolith® SpeedROD RP-18e	Merck Chromolith® FastGradient RP-18e	Supelco Ascentis® Express C18
Column type	Multilayered particle (5 µm) packed column	Classic monolithic column	Narrow monolithic column	Core-shell particle (2.7 µm) packed column
Column size (mm)	50 × 4.6	50 × 4.6	50 × 2	30 × 4.6
Column volume (mL)	0.55	0.77	0.39	0.51
Back pressure (PSI)	300–400	200–300	500–600	500–600
Peak symmetry	1.23–1.50	1.22–1.58	1.00–1.41	1.00–1.50
Peak resolution	3.75–6.06	3.41–8.16	2.65–10.38	2.36–15.34
Peak capacity	13.50–19.75	7.25–8.81	11.41–16.63	13.50–21.84

separation comparable with core–shell particle columns and higher then monolithic columns in SIC, based on the data in earlier work presented by our workgroup [6]. The method proved acceptable selectivity and sensitivity for analysis of tested kinds of food samples.

4.2 | Column properties

The multilayered particle column offers interesting capabilities for the SIC, it is stable within the changes of mobile phase composition and requires a minimum time for reequilibration when the flow is started/stopped; this feature is essential for the SIC with a discontinuous mobile phase flow. The chromatographic properties of the multilayered particle column were evaluated and compared to the properties of classic monolithic column, narrow bore monolithic column, and core–shell particle column with regard to the use in SIC technique. The chromatographic conditions were always the same and the separation parameters of all columns are summarized in Table 3. According to the results the columns were marked by points in Table 4 (1–5 points, 5 is the best) and depicted as a radar plots in Supporting Information Fig. S2. The key parameters for evaluation, which could be described by calculable values based on the separation were a backpressure, a peak symmetry, a peak capacity, and a column volume. The other important parameters, as producer choice of types of stationary phase chemistries, physical-chemical stability of stationary phase (pH, solvents, and pressure), typical column price, and overall analysis costs (regarding time of analysis and consumption of mobile phase) were evaluated using long-time authors experience (thus subjective). Total score described overall reliability of the column for the SIC. Both particle columns dominated, however lower scores of both monolithic columns do not limit their use in the SIC. Achieved results confirmed higher performance of multilayered particle packed column over classic monolithic column under similar work pressure (5 µm sized multilayered particles maintain the column porosity for lower backpressure and lower risk of clogging) and at the same time higher pH stability over monolithic and core–shell particle packed columns. The classic monolith is favorable by the lowest backpressure enabling the use in the SIC even with a glass syringe pump (or in related medium pressure flow methods) or use higher flow rates for reduction of separation time and quite broader choice of produced chemistries (recently presented new kinds: CN, NH_2, DIOL) than narrow monolith, but the other column features are significantly worse than in the competitors. The narrow monolith excels in a peak symmetry,

TABLE 4 Comparison of key parameters of four types of columns typically used in SIC (1–5 points, five is the best)

Parameter	YMC-Triart C18	Merck Chromolith® SpeedROD RP-18e	Merck Chromolith® FastGradient RP-18e	Supelco Ascentis® Express C18
Column back pressure	4	5	3	3
Peak symmetry	4	3	5	5
Separation performance	4	2	3	5
Column volume	3	2	5	4
SP chemistries	4	4	3	5
SP chemical stability	5	2	2	4
Column price	4	3	3	4
Analysis costs	4	4	3	3
Reliability	32	25	27	33

a low column volume, and correlating reduced analysis costs particularly to the lower mobile phase consumption, but narrow column diameter (and correlating backpressure) limits working flow rate in the SIC. The core–shell particle column proves high separation performance by excellent features that present trendy stationary phase technology, except the backpressure and physical-chemical stability that are worse than by multilayered particles.

5 | CONCLUSIONS

The developed SIC method demonstrates useful application of short (50 mm) chromatographic column and presents the high performance of modern columns based on current trends in the LC sorbents development. The multilayered particle packed column was compared in key parameters with monolithic and core–shell particle columns typically used in the SIC. This work demonstrates the next step in separation capabilities for medium-pressure flow methods and follows trends for developing techniques for fast chromatographic separations. The SIC manifold compared to the newest HPLC or UHPLC systems is much less sophisticated and therefore cheaper, however it can achieve high performance separations and determinations comparable to HPLC. Further, typical features of the SIC: simple and flexible manifold with low dead volume, easy operation, straightforward method development, discontinuous mobile phase flow, and easy hyphenation with on-line sample handling processes allow for advanced automation of analytical techniques.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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