



Assessment and Comparison of the Overall Analytical Potential of Capillary Electrophoresis and High-Performance Liquid Chromatography Using the RGB Model: How Much Can We Find Out?

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Abstract

The choice between capillary electrophoresis (CE) and high-performance liquid chromatography (HPLC) is not easy and depends on many factors. An attempt to facilitate this choice is this work, in which both techniques have been confronted on the basis of the RGB model, offering a transparent and pictorial way to compare individual parameters as well as the overall analytical potential of each tool. To ensure the universal nature of the comparison, a simple in composition and chemically diverse model sample was used, accompanied by the data processing method reducing the potential impact of analyte selection. Moreover, permanent coating of the inner surface of the capillary and addition of a surfactant to the separation buffer were considered as the additional factors that may affect the assessment of the CE technique. The presented analysis can be valuable in any discussions about the intrinsic advantages and disadvantages of CE and HPLC, and divergences on how they affect the overall potential and usability of each. We also provide access to the Excel worksheets used for the assessment, which can be easily modified to reevaluate the methods with a different selection of variables, and analyze other possible scenarios.

Keywords Capillary electrophoresis · High-performance liquid chromatography · Capillary coating · Micellar electrokinetic chromatography · RGB model

Introduction

Capillary electrophoresis (CE) and high-performance liquid chromatography (HPLC) are powerful and recognized in the analytical world separation techniques [1, 2], with different specifications, often mutually competitive due to different advantages and disadvantages. Despite the differences in the separation mechanism, in many cases, the separation of given groups of analytes is possible with good efficiency using both techniques. In such situations, choosing a better one in overall is not easy and requires an in-depth and

critical look. Any attempt to transparently compare their overall potential and usability requires analyzing various criteria, selecting them, assigning appropriate weights, and proposing an appropriate evaluation algorithm. The situation is complicated by the fact that in addition to the standardized validation criteria related to the quality of analytical results, the overall potential of the method is also determined by practical features, such as cost and time of analysis, sample consumption, complexity of the methodology and simplicity/convenience of use, as well as gaining in the last time more and more attention—compliance with the principles of green chemistry [3, 4].

The purpose of this work is to attempt to carry out a comprehensive assessment and comparison of CE and HPLC without the reference to a specific group of analytes, but instead using a chemically diverse model sample. For this purpose, we propose the use of a specialized evaluation algorithm, which has been recently proposed in the literature—the red–green–blue (RGB) model [5]. It allows to capture and analyze some specific features of both techniques, and find out how they affect their usability in a global

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perspective. We will try to answer the following questions: (i) Will the differences between the results of the assessment be large enough to indicate a clearly better tool in general?; (ii) In what areas will CE and HPLC be differently evaluated and in which equally?; (iii) How important for CE will be the coating of the inner surface of the capillary [6–10]?; (iv) Will the hybrid approach using micellar electrokinetic chromatography (MEKC) combine the advantages of electrophoresis and chromatography?. In our opinion, this work can be helpful in all choices between both techniques, and can provide valuable support when developing new methods based on CE and HPLC.

Materials and Methods

Materials

All analytes: nortriptyline (NT), paracetamol (PC), acetylsalicylic acid (ASA), and human apo-transferrin (TF) were supplied by Sigma-Aldrich (St. Louis, MO, USA). Stock solutions were prepared in the methanol (NT, PC, and ASA) or water (TF). Water was deionized using the MilliQ system (Merck-Millipore Billerica, MA, USA). The salts used for preparing buffers, and surfactant—tetradecyltrimethylammonium bromide (TTAB) were also supplied by Sigma-Aldrich. Other chemicals (organic solvents, washing reagents, mobile phase ingredients) were supplied by Avantor Performance Materials Poland. S.A. (Gliwice, Poland).

Sample

The model sample contained four chemically different molecules: NT—a small cationic molecule in the tested pH range, PC—a small electrically neutral molecule in the tested pH range, ASA—a small anionic molecule in the tested pH range, and TF—a globular water-soluble protein. The stock solutions were diluted in water, at the following final concentrations: NT, PC, and ASA – 0.48 mg mL⁻¹, TF – 0.43 mg mL⁻¹ (for CE), and all of them were ten times more diluted for HPLC. The dilution factor equaling to ten, applied for the HPLC method, was included in normalizing the sensitivity evaluation (signal-to-noise ratios obtained with HPLC were multiplied by ten).

CE Instrumentation

The PA 800 plus Capillary Electrophoresis instrument was used, (Beckman-Coulter, Brea, CA, USA) equipped with the UV–vis spectrophotometric detector. The unmodified bare fused-silica capillary and permanently coated amine (eCAP) capillary were used (Beckman-Coulter). They were of 60.0 cm total length, 50.0 cm effective length, and of

50 µm internal diameter. Between runs, the uncoated capillary was rinsed with 0.1 M NaOH for 1 min, and background electrolyte (BGE) for 2 min. Before the first use of the capillary at a working day: methanol for 5 min, 0.1 M HCl for 3 min, deionized water for 3 min, 0.1 M NaOH for 10 min, and BGE for 10 min were applied. For the fresh capillary conditioning, the latter sequence was used but the duration of each individual step was doubled. Between runs, the amine capillary was rinsed with deionized water for 1 min, and BGE for 2 min. Before the first use of the capillary at a working day: deionized water for 5 min, and BGE for 10 min were applied. For the fresh capillary conditioning, the latter sequence was used but the duration of each individual step was doubled. The pressure applied equaled to 137.9 kPa (20 psi). Sample injection was conducted using the forward pressure of 3.45 kPa (0.5 psi) for 5 s. During separations, the separation voltage of 30.0 kV (normal or reverse polarity, depending on the method tested) was applied, without the external pressure. The voltage ramp time was 0.2 min. The measured current values were below 50 µA. The temperature of cooling liquid was set at 25 °C. The detection and analysis were carried out at the wavelength of 210 nm, providing optimal signal-to-noise ratio. BGE was prepared by mixing 100 mM acetic acid and 100 mM sodium acetate, and by further dilution with the deionized water to 50 mM ionic strength. Its pH value was 5.0. In addition, to examine other method variants, BGE was enriched with the cationic surfactant – 25 mM TTAB, which in the tested conditions forms an admicelle layer coating on the inner capillary surface and reverses direction of electroosmotic flow (EOF) [11]. Therefore, this method variant should be considered as both capillary's surface modification and separation mechanism modification (micellar electrokinetic chromatography, MEKC). Each method variant was tested based on seven replicates performed one-by-one on the same day ($n=7$). All CE methods tested in this study were summarized in (Table 1). The different characteristics of the capillary inner surface studied herein are illustrated in (Fig. 1).

HPLC Instrumentation

In the case of HPLC method, the Agilent Technologies 1220 LC Infinity system was used (Santa Clara, CA, USA) equipped with UV–Vis spectrophotometric detector, and the Spheri-5 VL, RP, C18, 100×4.6 mm, 5 µm column. The mobile phase was used in the isocratic conditions, it was composed of solvent A—acetonitrile (60%), and solvent B (40%)—aqueous solution of orthophosphoric acid (0.14% v/v) and dimethylamine (0.10% v/v), pH 2.8. The temperature was set at 25 °C. Injection volume was 5 µL. The detection was performed at the wavelength of 210 nm, the same as for the CE-based methods. The same replicates number ($n=7$) was also maintained. Between runs, the column was

Table 1 Summary of all five methods tested in the present study

Method name	Instrument (technique)	Method type	Separation mechanism	Characteristics
CZE silica	CE	Capillary zone electrophoresis (CZE)	Electrophoretic	Uncoated silica capillary, cathodic electroosmotic flow, normal polarity applied
CZE amine	CE	Capillary zone electrophoresis (CZE)	Electrophoretic	Permanently coated amine capillary, anodic electroosmotic flow, reverse polarity applied
MEKC silica	CE	Micellar electrokinetic chromatography (MEKC)	Mixed (electrophoretic and chromatographic), due to surfactant addition forming pseudostationary phase	Silica capillary coated dynamically by surfactant molecules forming admicelle layer and reversing electroosmotic flow to anodic, reverse polarity applied
MEKC amine	CE	Micellar electrokinetic chromatography (MEKC)	Mixed (electrophoretic and chromatographic), due to surfactant addition forming pseudostationary phase	Permanently coated amine capillary, without surfactant layer due to repulsion of positive charges, anodic electroosmotic flow, reverse polarity applied
HPLC	HPLC	High-performance liquid chromatography	Chromatographic	Chromatographic separation in the reverse phase mode using a common C18 column

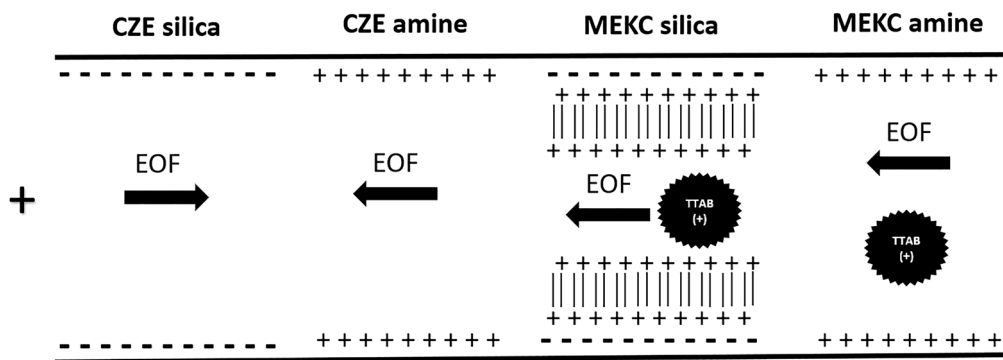


Fig. 1 Characteristics of the particular capillary inner surfaces in the CE-based methods; “+” and “-” symbols represent the positive and negative electric charges, respectively; *EOF* electroosmotic flow

rinsed with mobile phase for 3 min. Before the first use of the column at a working day: acetonitrile for 30 min, and mobile phase for 10 min were applied. The pressure values measured during separations were below 100 bars.

RGB Model

The RGB model for the evaluation of analytical methods was inspired by the concept of green analytical chemistry (assigning the label “green” to safe and environmentally friendly methods) and the RGB model of colors used commonly in electronics. A detailed description of the adaptation of the RGB model to the assessment of analytical methods, along with the justification of its rules, is in the original work to which we refer all interested readers [5]. According to this concept, as a result of evaluation carried out using a

simple Excel spreadsheet, the method acquires the final color resulting from the possession or absence of three primary colors assigned to three basic attributes: red—analytical performance (related to validation criteria), green—compliance with the rules green chemistry, and blue—practical and economic efficiency. To get the given primary color, its saturation rate—color score (CS), must be at least 66.6%. To facilitate the assessment, a simplified scheme of possible colors was proposed, shown in (Fig. 2). As one can see, in the ideal case the method is white, i.e., having concurrently all three primary colors. Otherwise, it can have two primary colors and be classified as magenta, yellow, or cyan, one primary color (red, green, blue), or none (gray, black). In the latter case, the method is black if at least one CS value is below 33.3%. In addition to the color, which determines the overall characteristics of the method in a qualitative way,

RESULTANT METHOD COLOR	REDNESS	BLUENESS	GREENNESS	GENERAL RECOMMENDATIONS
WHITE	CS \geq 66.6%	CS \geq 66.6%	CS \geq 66.6%	Method is complete and well-balanced in terms of all three main attributes (analytical performance, productivity/practical effectiveness, and safety/eco-friendliness). It is a good candidate for the method of choice for all applications.
MAGENTA	CS \geq 66.6%	CS \geq 66.6%	CS \geq 33.3%	Method provides a satisfactory score in the red and blue aspects, although it lacks the green character to be complete. It may be the method of choice if no "greener" alternatives are available.
YELLOW	CS \geq 66.6%	CS \geq 33.3%	CS \geq 66.6%	Method provides a satisfactory score in terms of the red and green aspects, although it lacks the blue character to be complete. It may be the method of choice if the number of planned analyzes is relatively low.
CYAN	CS \geq 33.3%	CS \geq 66.6%	CS \geq 66.6%	Method provides a satisfactory score in terms of the blue and green aspects, although it lacks the red character to be complete. It may be the method of choice if the requirements concerning quality of analytical result are less stringent.
RED	CS \geq 66.6%	CS \geq 33.3%	CS \geq 33.3%	Method is characterized by good analytical performance, although it provides only acceptable level of safety/eco-friendliness and productivity/practical effectiveness. It may be the method of choice if the number of planned analyzes is relatively low, and if no "greener" alternatives are available.
BLUE	CS \geq 33.3%	CS \geq 66.6%	CS \geq 33.3%	Method is characterized by good productivity/practical effectiveness, although it provides only acceptable level of analytical power and safety/eco-friendliness. It may be the method of choice if the requirements concerning quality of analytical result are less stringent, and if no "greener" alternatives are available.
GREEN	CS \geq 33.3%	CS \geq 33.3%	CS \geq 66.6%	Method is generally safe and eco-friendly, although it provides only acceptable level of analytical power and productivity/practical effectiveness. It may be the method of choice if the number of planned analyzes is relatively low, and if the requirements concerning quality of analytical result are less stringent.
COLORLESS (GRAY)	CS \geq 33.3%	CS \geq 33.3%	CS \geq 33.3%	Method is generally acceptable in all aspects, although it lacks clear predispositions. Its utilization may be conditionally considered, if no better methods are available.
BLACK	CS $<$ 33.3% for one or more primary attributes (lack of acceptance)			Appropriate method utilization is doubtful because it is defective on account of one or more primary attributes, and this overshadows any positive features.

Fig. 2 Nine resultant colors of a method predicted by the RGB model, depending on the respective CS values, and resulting general recommendations

the model also offers a parameter for the overall quantitative assessment—method brilliance (MB, 0–100%). MB is calculated as the weighted geometric mean of CS values for the given primary colors, where the weights (W) determine the significance of a given primary attribute (red, green and blue), depending on the adopted assessment rules.

The assessment algorithm begins with the selection of a few criteria that are most relevant in a given case, belonging to the red, green and blue attributes, and assigning them weights (w) determining their relative significance. The sum of weights of all the criteria in a given color is ten. Then, the given criterion is evaluated, for example precision belonging to the red area expressed by the RSD value, using the Score parameter from 0 to 100, with the possibility of rounding the assigned values for simplification. This assessment is made in relation to the previously defined reference values (in this case RSD limits), which indicate the lowest acceptable value (LAV)—tolerance threshold, adequate for the value of Score = 33.3, and lowest satisfactory value (LSV)—satisfaction threshold, adequate for the value of Score = 66.6. CS values for a given primary color are calculated as a weighted geometric mean of the Score values, included with the weights " w ". The model's variables, i.e. selection of criteria and their weights, reference values LAV and LSV, and weights of given primary attributes (w) can be

determined subjectively by the evaluator, or in a more objective way, based on generally accepted rules imposed from above. To facilitate the assessment, an Excel spreadsheet has been designed with ready-made assessment templates, programmed calculation formulas, and conditional formatting functions, also available as an attachment to this work.

Algorithm Specification

To compare CE and HPLC techniques in the most general and comprehensive way possible, the criteria were selected that depend as little as possible on the composition of the sample, and more on the very specifics of the technique. To this end, the assessment of the efficiency of individual peaks separation was abandoned (as resolution strictly depends on analyte selection), and additionally, a rule was adopted according to which the final result of the method, e.g., repeatability measured by the RSD value for migration/retention times, is calculated as the arithmetic mean of the three best values among the four analytes present in the sample. Averaging allowed to minimize the dependence of the measured parameter on the chemical nature of the analyte, while rejection of the fourth weakest result allowed the minimization of the risk that any of the tested methods will prove to be highly inadequate for a given analyte (optimization

of individual methods was not performed to not introduce additional variables between methods).

The “red” criteria included: repeatability of migration or retention times ($n=7$), with weight $w=4$; number of theoretical plates as a measure of the overall capillary/column efficiency, with weight $w=3$; and sensitivity measured as the ratio of peak height to noise corresponding to a normalized analyte concentration, with weight $w=3$. In our opinion, these three criteria capture the most important aspects that may determine the analytical efficiency of a method, and contain features often cited as shortcomings of given techniques, e.g., weaker repeatability of migration times and sensitivity for CE (associated with EOF fluctuations and short optical path), and lower number of plates for HPLC (associated with increased dispersion in comparison to electrokinetic techniques). The “green” criteria included: total amount of waste, with the weight $w=4$; total toxicity of the reagents measured by the total number of pictograms, with the weight $w=3$; other occupational hazards, with weight $w=2$; and electric energy consumption, with weight $w=1$. The “blue” criteria included: time of analysis, with the weight $w=3$; cost of analysis, with weight $w=3$; volume of the introduced sample, with weight $w=2$; and ‘other aspects’, with the weight $w=2$. ‘Other aspects’ were assessed in an intuitive and qualitative manner, taking into account factors such as simplicity and convenience of use, theoretical complexity and estimated risk of technical problems, e.g. capillary-related problems or electric current instability in the case of CE. We have found that these criteria are particularly important in assessing greenness and blueness of any separation methods, taking into account their specification and potential expectations of users. Their weights were assigned based on numerous discussions held within the forum of our department’s representatives. The exact algorithms of assessing the green and blue criteria are presented in the Electronic Supplementary Material (ESM). In the case of criteria depending on the number of analyzes performed, the number of 100 model replicates carried out under the same conditions was assumed, with the possible need to prepare fresh reagents and appropriate capillary/column rinsing during experiment (waste amount, cost, time and sample consumption refer to 100 separations).

The red attribute (analytical performance) was treated with the highest relative weight $w=5$, which according to our experience, reflects the analysts’ general expectations that an effective method should primarily ensure a good quality of the analytical result. The blue attribute (productivity and practical efficiency) was treated as the second most important, with the relative weight $w=4$, because as we assume, the next general expectation of analysts relates to the practical aspects of the analytical procedure, which can often be another limiting factor. The green attribute (compliance with the principles of green analytical chemistry, i.e.,

environmental friendliness and safety) was treated with the weight $w=3$. This choice still reflects the strong emphasis on “greenness”, but does not give it priority or equal significance to the red or blue attributes, which may seem more important.

Results

Algorithm Visualization

The exemplary electropherograms and chromatograms obtained for the model sample during the study are shown in (Fig. 3). The whole results of the method evaluation were presented in the form of special tabular schemes, (Figs. 4, 5, 6, 7, 8.), obtained using a programmed Excel spreadsheet mentioned before (see ESM). The selected criteria are represented by the merged cells, the weight of the given criterion within the considered color (w) corresponds to the number of merged columns, while the weight of a given primary color (w) corresponds to the number of merged rows. Thus, the larger the cell, the more important it is in assessing the whole method. Its color (black, gray or red/green/blue) indicates whether the result obtained is or is not within the reference ranges defined by LAV and LSV (the result worse than LAV gives black, the result worse than LSV gives gray, while better than LSV, red/green/blue). Score values assigned by the evaluator follow the relationship between result and LAV/LSV, using a scale of 0–100, and are entered in each column (“ w ” times). The final color of the method and the MB value is calculated automatically and are provided at the bottom of the table (for more information on the interpretation of the algorithm, see [5]). Summary of the most important quantitative results of the assessment, CS and MB values, is presented in (Fig. 9).

HPLC versus Classical CE (CZE silica)

At the beginning, HPLC and CZE silica methods will be compared, i.e. the basic CE variety using a typical uncoated capillary and no surfactant added. As shown in (Figs. 4, 8, 9), the individual criteria were assessed quite differently. HPLC significantly exceeds CZE silica in terms of CSred (analytical performance), 72.8% vs. 50.5%, which results from worse repeatability and sensitivity of electrophoresis. This result allowed to obtain the “red” attribute by HPLC (CSred above 66.6%, Fig. 2). However, the number of theoretical plates was assessed more favorably for CZE silica, allowing to maintain CSred above 50%. These observations are consistent with the generally prevailing belief as to the advantages and disadvantages of each technique [12–16], resulting from the specifics of the separation mechanism (repeatability, number of plates) and typically technical

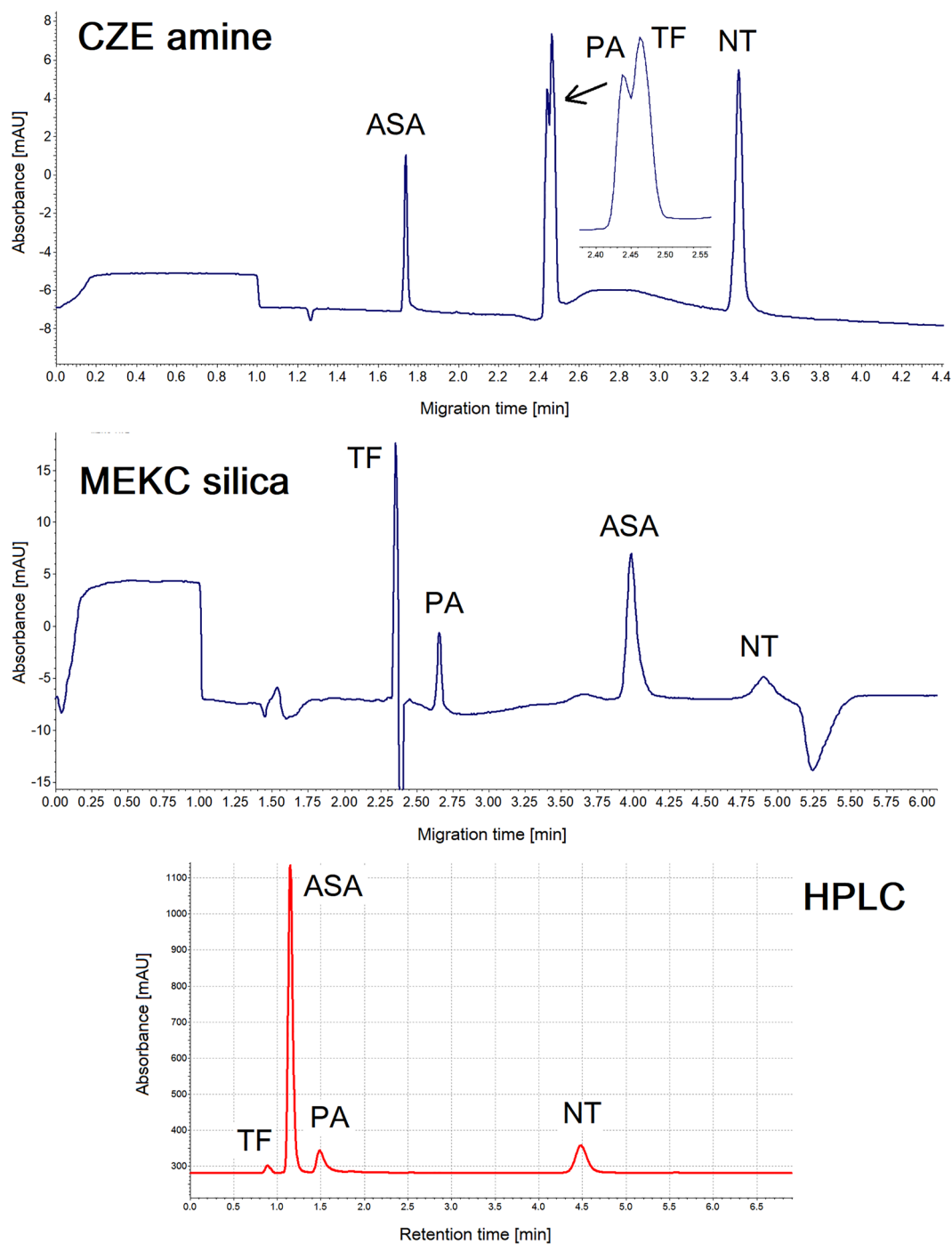


Fig. 3 Exemplary electropherograms (blue) and chromatogram (red) obtained for the model sample, presented for the three best-rated methods. The overlapped peaks (CZE amine) were deconvoluted to assess theoretical plate number

issues, such as optical path length (sensitivity). The value of CSgreen (eco-friendliness and safety) is significantly better for CZE silica, 52.0% vs. 69.3%, which allowed it to obtain a green primary color. This time the difference between the methods results from only one criterion, the amount of generated waste, which is significantly higher in the case of

chromatography due to the larger diameter of columns and total flow. The toxicity of the reagents used, and the energy consumption are similar for both techniques, while HPLC is more favorable in terms of additional hazards (for CE there are two: high voltage and risk of capillary injury, for HPLC there is one: contact with solvent vapors). The CSblue value

CZE silica														
REDNESS (analytical performance)		W=5	w=4				w=3			w=3				
			Repeatability (RSD)				Theoretical plates number			Sensitivity (signal-to-noise)				
			CS: 50.5%		LAV=33.3	5%			500			100		
					LSV=66.6	1%			5 000			500		
Result	3.59%				14 100			118						
		Score (0-100)	45	45	45	45	85	85	85	35	35	35		
GREENNESS (safety and eco-friendliness)		W=3	w=4				w=3			w=2		w=1		
			Waste amount				Toxicity of chemicals			Other hazards		Energy intake		
			CS: 69.3%		LAV=33.3	2 000 mL			15 pictograms			7 hazards		50 kVAh
					LSV=66.6	200 mL			5 pictograms			2 hazards		10 kVAh
Result	60 mL				10 pictograms			2 hazards		9 kVAh				
		Score (0-100)	90	90	90	90	50	50	50	66.6	66.6	70		
BLUENESS (productivity / practical effectiveness)		W=4	w=3				w=3			w=2		w=2		
			Time of analysis				Cost of analysis			Sample consumption		Other aspects		
			CS: 65.6%		LAV=33.3	100 h			1 500 PLN (ca. 375 USD)			5 mL		acceptable
					LSV=66.6	30 h			300 PLN (ca. 75 USD)			0.5 mL		satisfactory
Result	34 h				601 PLN (ca. 150 USD)			2.5 µL		moderate				
		Score (0-100)	65	65	65	65	60	60	60	100	100	50	50	
FINAL COLOR:			REDNESS		GREENNESS		BLUENESS			BRILLIANCE (MB):		59.6%		
GREEN			≥33.3%	≥66.6%	≥33.3%	≥66.6%	≥33.3%	≥66.6%	yes				no	
Short annotation: 59.6green			Long annotation: 59.6green(50.5/5red-69.3/3green-65.6/4blue)											

Fig. 4 Outcomes of the CZE silica method evaluation using the RGB algorithm

CZE amine														
REDNESS (analytical performance)		W=5	w=4				w=3			w=3				
			Repeatability (RSD)				Theoretical plates number			Sensitivity (signal-to-noise)				
			CS: 69.3%		LAV=33.3	5%			500			100		
					LSV=66.6	1%			5 000			500		
Result	1.25%				27 000			325						
		Score (0-100)	65	65	65	65	95	95	95	55	55	55		
GREENNESS (safety and eco-friendliness)		W=3	w=4				w=3			w=2		w=1		
			Waste amount				Toxicity of chemicals			Other hazards		Energy intake		
			CS: 77.2%		LAV=33.3	2 000 mL			15 pictograms			7 hazards		50 kVAh
					LSV=66.6	200 mL			5 pictograms			2 hazards		10 kVAh
Result	50 mL				5 pictograms			2 hazards		9 kVAh				
		Score (0-100)	95	95	95	95	66.6	66.6	66.6	66.6	66.6	70		
BLUENESS (productivity / practical effectiveness)		W=4	w=3				w=3			w=2		w=2		
			Time of analysis				Cost of analysis			Sample consumption		Other aspects		
			CS: 53.7%		LAV=33.3	100 h			1 500 PLN (ca. 375 USD)			5 mL		acceptable
					LSV=66.6	30 h			300 PLN (ca. 75 USD)			0.5 mL		satisfactory
Result	30 h				1 794 PLN (ca. 450 USD)			2.5 µL		moderate				
		Score (0-100)	66.6	66.6	66.6	30	30	30	100	100	50	50		
FINAL COLOR:			REDNESS		GREENNESS		BLUENESS			BRILLIANCE (MB):		65.4%		
YELLOW			≥33.3%	≥66.6%	≥33.3%	≥66.6%	≥33.3%	≥66.6%	yes				no	
Short annotation: 65.4yellow			Long annotation: 65.4yellow(69.3/5red-77.2/3green-53.7/4blue)											

Fig. 5 Outcomes of the CZE amine method evaluation using the RGB algorithm

MECK silica													
				w=4				w=3			w=3		
REDNESS (analytical performance)		W=5		Repeatability (RSD)				Theoretical plates number			Sensitivity (signal-to-noise)		
CS: 79.6%		LAV=33.3	5%				500			100			
		LSV=66.6	1%				5 000			500			
		Result	0.09%				15 700			393			
		Score (0-100)	100	100	100	100	85	85	85	55	55	55	
GREENNESS (safety and eco-friendliness)		W=3		Waste amount				Toxicity of chemicals			Other hazards		Energy intake
CS: 67.1%		LAV=33.3	2 000 mL				15 pictograms			7 hazards		50 kVAh	
		LSV=66.6	200 mL				5 pictograms			2 hazards		10 kVAh	
		Result	60 mL				11 pictograms			2 hazards		9 kVAh	
		Score (0-100)	90	90	90	90	45	45	45	66.6	66.6	70	
BLUENESS (productivity / practical effectiveness)		W=4		Time of analysis				Cost of analysis			Sample consumption		Other aspects
CS: 64.1%		LAV=33.3	100 h				1 500 PLN (ca. 375 USD)			5 mL		acceptable	
		LSV=66.6	30 h				300 PLN (ca. 75 USD)			0.5 mL		satisfactory	
		Result	36 h				627 PLN (ca. 157 USD)			2.5 µL		moderate	
		Score (0-100)	60	60	60	60	60	60	60	100	100	50	50
FINAL COLOR:				REDNESS		GREENNESS		BLUENESS		BRILLIANCE (MB):		71.0%	
YELLOW				≥33.3%	≥66.6%	≥33.3%	≥66.6%	≥33.3%	≥66.6%				
				yes	yes	yes	yes	yes	no				
Short annotation: 71.0yellow				Long annotation: 71.0yellow(79.6/5red-67.1/3green-64.1/4blue)									

Fig. 6 Outcomes of the MEKC silica method evaluation using the RGB algorithm

MECK amine													
				w=4				w=3			w=3		
REDNESS (analytical performance)		W=5		Repeatability (RSD)				Theoretical plates number			Sensitivity (signal-to-noise)		
CS: 51.2%		LAV=33.3	5%				500			100			
		LSV=66.6	1%				5 000			500			
		Result	5.09%				5 700			535			
		Score (0-100)	33.3	33.3	33.3	33.3	70	70	70	66.6	66.6	66.6	
GREENNESS (safety and eco-friendliness)		W=3		Waste amount				Toxicity of chemicals			Other hazards		Energy intake
CS: 74.8%		LAV=33.3	2 000 mL				15 pictograms			7 hazards		50 kVAh	
		LSV=66.6	200 mL				5 pictograms			2 hazards		10 kVAh	
		Result	50 mL				6 pictograms			2 hazards		9 kVAh	
		Score (0-100)	95	95	95	95	60	60	60	66.6	66.6	70	
BLUENESS (productivity / practical effectiveness)		W=4		Time of analysis				Cost of analysis			Sample consumption		Other aspects
CS: 52.0%		LAV=33.3	100 h				1 500 PLN (ca. 375 USD)			5 mL		acceptable	
		LSV=66.6	30 h				300 PLN (ca. 75 USD)			0.5 mL		satisfactory	
		Result	36 h				1 864 PLN (ca. 466 USD)			2.5 µL		moderate	
		Score (0-100)	60	60	60	30	30	30	100	100	50	50	
FINAL COLOR:				REDNESS		GREENNESS		BLUENESS		BRILLIANCE (MB):		56.6%	
GREEN				≥33.3%	≥66.6%	≥33.3%	≥66.6%	≥33.3%	≥66.6%				
				yes	no	yes	yes	yes	no				
Short annotation: 56.6green				Long annotation: 56.6green(51.2/5red-74.8/3green-52.0/4blue)									

Fig. 7 Outcomes of the MEKC amine method evaluation using the RGB algorithm

HPLC												
REDNESS (analytical performance)		W=5	w=4				w=3			w=3		
			Repeatability (RSD)				Theoretical plates number			Sensitivity (signal-to-noise)		
			5%				500			100		
			1%				5 000			500		
CS: 72.8%		LAV=33.3	0.24%				803			30 643		
		LSV=66.6					40			40		
		Result					100			100		
		Score (0-100)	90	90	90	90	40	40	40	100	100	
GREENNESS (safety and eco-friendliness)		W=3	w=4				w=3			w=2		w=1
			Waste amount				Toxicity of chemicals			Other hazards		Energy intake
			2 000 mL				15 pictograms			7 hazards		50 kVAh
			200 mL				5 pictograms			2 hazards		10 kVAh
CS: 52.0%		LAV=33.3	1 600 mL				10 pictograms			1 hazard		9 kVAh
		LSV=66.6					50			50		
		Result					80			80		
		Score (0-100)	40	40	40	40	50	50	50	80	70	
BLUENESS (productivity / practical effectiveness)		W=4	w=3				w=3			w=2		w=2
			Time of analysis				Cost of analysis			Sample consumption		Other aspects
			100 h				1 500 PLN (ca. 375 USD)			5 mL		acceptable
			30 h				300 PLN (ca. 75 USD)			0.5 mL		satisfactory
CS: 58.8%		LAV=33.3	35 h				1050 PLN (ca. 263 USD)			0.5 mL		satisfactory
		LSV=66.6					65			65		
		Result					45			45		
		Score (0-100)	65	65	65	45	45	45	66.6	66.6	66.6	
FINAL COLOR:			REDNESS		GREENNESS		BLUENESS		BRILLIANCE (MB):		62.3%	
RED			≥33.3%	≥66.6%	≥33.3%	≥66.6%	≥33.3%	≥66.6%				
			yes	yes	yes	no	yes	no				
Short annotation: 62.3red			Long annotation: 62.3red(72.8/5red-52.0/3green-58.8/4blue)									

Fig. 8 Outcomes of the HPLC method evaluation using the RGB algorithm

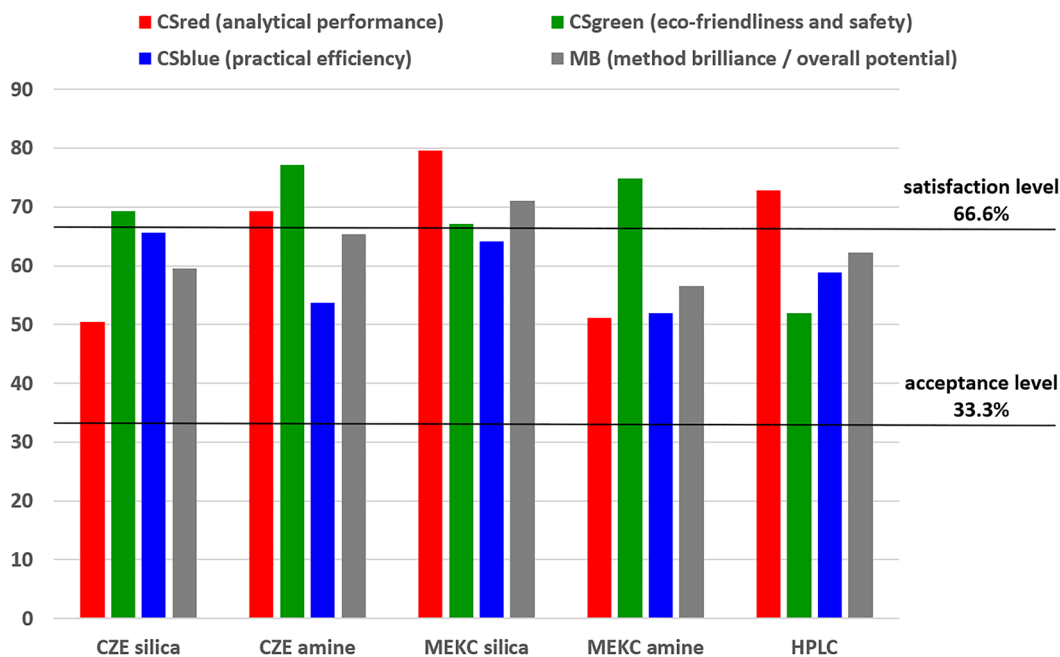


Fig. 9 Comparison of the main evaluation outcomes obtained for the particular methods

(practical and economic issues) proved to be better for CZE silica, 58.8% vs. 65.6%, but still slightly below the satisfaction threshold (66.6%, Fig. 2) required to get blue color. CZE

silica was rated better in terms of the cost of analysis and sample volume introduced. All detailed point-by-point cost estimates are shown in ESM. As it can be seen, the lower

cost effectiveness of HPLC mainly comes from the need to purchase large quantities of high purity acetonitrile used as the mobile phase component. The much lesser amount of sample needed for injection is, in turn, an intrinsic advantage of the CE instrument specification. The analysis time comprising the whole analytical procedure, including also other mandatory steps than separation, turned out to be very similar (see the exact estimations in ESM), while 'other aspects' were rated more favorably for HPLC. This is due to the more reliable nature of HPLC, a lower risk of technical problems (capillary breakage and current instability are quite common for CE), and the methodology that seems a bit easier in both theoretical and practical terms.

As a result, the final HPLC color is red, while CZE silica is green. These colors clearly indicate which attributes are the strengths of a given technique as a whole, and where to look for possible shortcomings and areas for improvement (Fig. 2). The MB value, being a quantitative measure of the potential of a given method taking into account the weights assigned ($w = 5, 3, 4$ for R, G, and B, respectively), turned out to be slightly more favorable for HPLC, 62.3% vs. 59.6%. Thus, the RGB model indicates the HPLC method as slightly better in general.

Permanent Capillary Coating

The eCAP amine capillary is characterized by a stable positively charged coating that causes reversal, stabilization, and often, increase of EOF [12]. As it can be seen from the comparison of (Figs. 4, 5), and directly from (Fig. 9), the CZE amine method using this type of capillary looks significantly more favorably than CZE silica. The only serious drawback of the CZE amine method is the cost of analysis (marked in black in Fig. 7 due to the Score < 33.3), which indicates that this is a main limitation of this method. As shown in ESM, this is due to the expensive capillary purchased from a commercial source, about ten times more costly than the ordinary uncoated silica capillary. Nevertheless, this surface modification improved the red parameters (repeatability, number of plates, sensitivity), but also green ones (waste amount and reagent toxicity) and blue one (analysis time). The effect observed in the green and blue areas results from the reversal and increase of EOF (at the same absolute voltage), and thus the shortening of the separation time, as well as the simpler and faster capillary rinsing procedure, which in addition eliminates the use of some chemicals (methanol, HCl, NaOH). As a result, the CZE amine method obtained the resultant yellow color (concurrently red and green), and the high MB value, 65.4%, exceeding not only CZE silica, but also HPLC. It is worth noting that the MB value was actually significantly lowered by the poorly assessed cost of analysis, and consequently, the low value of CSblue. It is, therefore, a clear indication of where to look for potential solutions that may increase the utility of this

method, e.g., by carrying out the capillary coating procedure in laboratory.

Addition of Surfactant

The addition of TTAB (MEKC silica method) caused a modification of two types: the formation of positively charged micelles that can be considered as a pseudostationary phase responsible for the mixed separation mechanism (a combination of electrophoresis and chromatography, see Table 1), as well as a dynamic modification of the inner surface of the capillary, resulting in the formation of a positive TTAB admicelle layer and reversing of EOF direction [11] (Fig. 1). As shown in (Figs. 6, 9), this modification significantly increased CSred to nearly 80%, even above the value obtained for HPLC (72.8%). This change results from an excellent repeatability of migration times (RSD < 0.1%), a high number of theoretical plates, and good sensitivity (though worse than for HPLC). Compared to CZE silica, the MEKC silica method was assessed a bit less favorably in the green and blue areas, which resulted in a slight decrease in CSgreen and CSblue. This is associated with the slightly worse rated toxicity (TTAB as an additional reagent), and the slightly longer analysis time resulting from a different separation mechanism. Nevertheless, the overall evaluation of the MEKC silica method is very positive, it is characterized by yellow color, like CZE amine, and the only MB value among all methods exceeding 70%.

Combined Modifications

It is worth noting that the MEKC amine method, which is a combination of both tested modifications (the use of permanently coated amine capillary and the addition of TTAB) did not cause significant improvements compared to the basic CZE silica method (Figs. 4, 7, 9). Its CSred value turned out to be similar, while CSblue worse than for the compared reference method. This is mainly due to the poor repeatability, which suggests that EOF under the tested conditions was quite unstable, and the high cost of analysis raised by the amine capillary. Explanation of the phenomenon of decline in repeatability is currently not possible, although it can be assumed that it is associated with the specific nature of the interaction of TTAB molecules with a double electrical layer on the surface of the silica capillary. The MB value of the MEKC amine method turned out to be the weakest of all methods, 56.6%, which places it last one in the ranking.

Discussion

In summary, no method has obtained a white color indicating full consistency and completeness. The closest to the ideal turned out to be CZE amine and MEKC silica, i.e. the

modified versions of CE. Their yellow color indicates both good analytical performance and environmental friendliness. The HPLC method, classified as red, compares unfavorably to CE in terms of the waste amount, and this fact does not allow it to be called a “green” method. CZE silica (classic CE version) and MEKC amine (combined modifications) were classified as “only green”, and in turn, do not deserve to be called “red” (of high analytical performance).

The obtained MB values indicate the MEKC silica method as the winner of the ranking (71.0%), mainly due to the significantly improved analytical performance compared to CZE silica, concurrent maintaining of the good environmental friendliness, and still the low cost of analysis. The CZE amine method (MB = 65.4%) clearly stands out from MEKC silica due to the elevated cost of analysis by expensive capillary. The HPLC method (MB = 62.3%) takes the middle position in the ranking, despite the best sensitivity among all tested methods, its position could not be better mainly due to the relatively low number of theoretical plates, large waste amount, significantly larger volume of introduced samples, and costs of analysis exceeding the CE methods based on a bare capillary. The classic CE method (CZE silica) was ranked behind HPLC (MB = 59.6%), mainly due to the large difference in CS_{red}. The ranking closes with the MEKC amine method, with the value of MB = 56.6%, which seems to be a rather unfortunate combination of two modifications.

Thus, this analysis suggests that the overall potential of the basic CE variant and HPLC is quantitatively similar, and the choice of clearly better one is not straightforward. It depends on the weights of the individual criteria, which should be adequately fitted to the planned application. The increase in the global potential of CE, observed after applying the proposed modifications (CZE amine and MEKC silica methods), clearly indicates them as noteworthy alternatives to the classic version of the technique. In particular, the MEKC silica method, which is more cost effective than CZE amine, seems to nicely combine the possibilities of CE and HPLC, which may confirm its hybrid nature between electrophoresis and chromatography in a positive sense.

It should be noted, however, that the present analysis is only an attempt to compare the general possibilities of CE and HPLC, and thus the results obtained should be considered only as some supporting indication. For example, no modification of the HPLC technique was tested, and furthermore, despite the efforts made, the assessment results could still be somewhat dependent on the choice of model analytes. For example, the presence of protein could, to some extent, adversely affect repeatability and sensitivity of CE due to interaction with the capillary inner surface. In addition, one should be aware of the importance of selecting the RGB model variables, such as particular criteria, their weights, and reference values (LAV and LSV). These variables have

been chosen in a subjective and intuitive way. A noteworthy possibility of minimizing the subjectivity of this test is to consider other scenarios, a different set of model’s variables or another set of experimental data, and then analyze the discrepancies received. To simplify this option, the original Excel worksheets used for the present assessment (with the appropriate formulas and formatting coded) are attached to this paper (ESM), which anyone interested can easily modify and adapt according to own preferences.

Conclusions

The general “power” of the basic CE variant and HPLC is quite similar. It was estimated with the model MB scale at around 60%, thus, it is still far from the assumed ideal case. The presented analysis based on the RGB model, supported by the detailed evaluations of some commonly overlooked parameters, was helpful in providing a transparent information in which aspects the tested methods offer similar possibilities, and in which differ and may constitute mutually complementary tools. A huge role of modifications such as the use of coated capillary and addition of surfactant to separation buffer has been demonstrated for the CE-based methods. They proved to be an effective way to improve the quality of analytical results obtained using the CE instrument, while maintaining environmental friendliness associated with low waste production. This was illustrated by the acquisition of a yellow color by these methods. The MEKC mode, especially, was able to increase the MB index from 59.5% to 71%, making a large step toward the ideal situation. Although this analysis does not provide fully objective and unambiguous answers about superiority of CE or HPLC, and the choice of a better method will always depend on the specifics of the sample itself and the associated experimental conditions, the proposed evaluation algorithm can be a valuable auxiliary tool. In particular, it can be a good global “measure” of the optimization process of newly developed method, encourage for more critical and in-depth method evaluations, as well as offer a convenient way to find the optimal solution in difficult situations, requiring more thorough analysis, when our intuition can fail. We believe that this work can be usable especially by providing a general guideline for using the RGB algorithm, as well as the completed Excel spreadsheets that can be modified and utilized in any kind of similar assessments.

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Compliance with Ethical Standards

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