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Examination of colour inkjet printing inks by capillary electrophoresis

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ABSTRACT

The possibility of comparing inkjet printing inks by micellar electrokinetic capillary electrophoresis (MECC) with diode array detection was studied. An analytical procedure was designed and successfully applied to discriminate between the electrophoretic profiles of inks (extracted from paper) produced by five well-known manufacturers. The separation process was conducted in a polyimide-coated fused silica capillary (ID 50 μm, 60 cm total/50 cm effective length) with +30 kV high voltage applied. Background electrolyte was used of the following optimum composition: 40 mM sodium borate buffer, 20 mM sodium dodecyl sulphate(IV) (SDS) and 10% (v/v) acetonitrile (pH 9.56). The experimental conditions were adjusted in terms of resolution and analysis time. The best results were obtained at 10 and 25 °C storage and capillary temperature, respectively, using 25 dots (ø 0.8 mm) cut from printouts as the sample and BGE diluted with water (1:99, v/v) as the injecting solution. The MECC separation of main printing ink components by the proposed method showed excellent precision – the RSD value of the migration time calculated for each of the investigated peaks did not exceed 3.3%. The optimized method was applied to group identification and differentiation of: (a) three colours of printing inks, (b) inks from different manufacturers (Hewlett–Packard, Epson, Brother, Lexmark and Canon) and (c) inks from different printer models. In all these cases, inks were successfully differentiated on the basis of position (migration time) and shape of their characteristic peaks.

1. Introduction

Nowadays, electronic communication, electronic signatures and a whole array of electronic business transactions are becoming increasingly prevalent. Nevertheless, documents printed on paper remain widely used and, moreover, due to widespread use of personal computers, forensic analyses of inkjet printing inks are increasingly requested. In 2008, about 59% of Polish homes owned a computer, compared to about 36% in 2004. Inkjet printers accompany many of these personal computers and even more are present in business offices. In addition, technological development has led to the rapid expansion of the range of printers and ink compositions used all over the world.

Printers are frequently used in forgery – the process of making, adapting or imitating documents with intent to deceive. Forensic experts are encountering ever more complex and demanding analytical problems because of the sophisticated evolution of printing technologies. The huge demand for specialized printing instruments has resulted in a rise in the number of ink formulations, depending on the intended function. The wide array of chemical components contained in inkjet printing inks, coupled with the possibility of contamination from the carrier, as well as chemical ageing of inks, make it necessary to use separation techniques for the analysis of such complex mixtures. To make the problem more challenging, the only available information about particular inkjet printing inks composition is included in Material Safety Data Sheets (MSDS). What is more, only in the Hewlett–Packard and Canon MSDS is the chemical class of used dyes provided. In Brother, Epson and Lexmark MSDS, this information is a trade secret.

Review articles regarding this issue, describing achievements in this region of research published up to 2007, reported that the comparison and differentiation of inks can be carried out by several non-destructive analytical methods (e.g. optical and microscopic techniques, Raman spectroscopy) and a great number of destructive ones, such as: spectrophotometric (UV–vis, FTIR), chromatographic (TLC, HPLC, GC, GC–MS, py-GC) and electrophoretic (CE) [1–7].

Capillary electrophoresis (CE) is an excellent option for this purpose, due to its many advantages over traditional modes of separation. Sample requirements are especially attractive from the forensic point of view. Only very small quantities of material are needed for analysis, meaning that there is minimal destruction and thus the integrity of the questioned document is maintained. Furthermore, CE has great resolution power and low reagent

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Table 1
Examples of previously developed procedures for CE analysis of inks and dyes.

Analyzed material	Sample taken	Extracting agent	BGE	CE technique	Ref.
Inkjet printers	5 dots (ID 0.45 mm)	2 mM Brij-35: MEE (2:1, v/v)	60 mM SDS, 15 mM 3-AP, 7.5 mM HCl, 0.5 mM Brij-35 + 30% (v/v) ACN	MECC	[11]
Ballpoint pens	5 dots (ID 0.45 mm)	2 mM Brij-35: MEE (2:1, v/v)	60 mM SDS, 15 mM 3-AP, 7.5 mM HCl, 0.5 mM Brij-35 + 30% (v/v) ACN	MECC	[11]
	10 dots (ID 1.5 mm)	ACN: BGE (1:1, v/v)	50 mM sodium borate pH 0.9 + 30 mM SDS + 50% (v/v) ACN	MECC	[12]
	6 dots (ID 0.8 mm)	Pyridine: water (1:1, v/v)	42 mM SDS, 10.5 mM 3-aminopropanol, 5.25 mM HCl, 0.35 mM Brij-35 + 30% (v/v) ACN	MECC	[13]
	5 dots (ID 1.0 mm)	MeOH	*Cationic dyes*: 25 mM sodium acetate + 25 mM glacial acetic acid + 10 mM CTAB + 30% (v/v) MeOH *Anionic dyes*: 25 mM CHES + 5 mM β-cyclodextrin	CE	[14,15]
	Directly from pen	No extraction	60 mM SDS + 30 mM H_3PO_4 in H_2O/ACN/MeOH (80:10:10, v/v/v)	MECC	[16]
Fountain pens	6 dots (ID 1.6 mm)	EtOH: water (1:1, v/v)	100 mM sodium borate (pH 8.0) + 20% (v/v) MeOH	CZE	[17]
	10 dots (ID 1.5 mm)	MeOH: 5 mM sodium borate (1:1, v/v)	100 mM sodium borate (pH 8.0) + 20% MeOH	CZE	[18]
Rollerball pens	5 mm of writing line	28% ammonia: EtOH (1:1, v/v)	15 mM sodium borate (pH 8.8)	CZE	[19]
Fiber tip pens	1 cm of writing line	Oxalium (0.5%)	20 mM sodium borate (pH 9.0)	CZE	[20]
Gel pens	Directly from pen	No extraction	0.1 M ammonium acetate (pH 4.5) + 25% (v/v) MeOH	CZE	[21]
	Directly from pen	No extraction	100 mM sodium borate + 25 mM SDS	CZE	[22]
Dyes	5 mg of powder/ 25 mL methanol	No extraction	20 mM ammonium carbonate (pH 9.0)	CE-MS	[23]
	Lack of data	0.1 M SDS	40 mM sodium tetraborate (pH 9.25)	CZE	[24]
	Lack of data	1 M sulphuric acid + dichloromethane (step2)	20 mM sodium tetraborate (pH 9.0) + 20 mM SDS + 10% (v/v) ACN	MECC	[25]
Other inks	Lack of data	0.1 M SDS	20 mM sodium tetraborate (pH 9.2)	CZE	[26]

consumption. Unlike many other analytical methods, the basic instrumental configuration for CE can be adapted – by varying mode – to a wide variety of analyses without great cost or difficulty. While in CZE (Capillary Zone Electrophoresis) – the simplest mode of CE – only charged analytes can be separated, MECC (Micellar Electrokinetic Capillary Chromatography) enables simultaneous separation of chemically different substances, ranging from small to large, charged to neutral or even very hydrophobic particles. This is possible due to adding surfactants and organic solvents to the BGEs. As a consequence of the mechanism, which is based on the affinity of the given analyte for the micelles which are created by the surfactant, MECC provides several peaks of neutral compounds instead of just one peak.

However, application of CE to inks analysis, especially for forensic purposes, encounters many specific problems. A poor CE detection limit – a disadvantage caused by a short optical light path in capillaries – can be improved by application of a phenomenon called sample stacking, defined by Mala [8] as 'on-line concentration of diluted analytes'. The simplest technique from this group – normal stacking mode (NSM), based on hydrodynamic injection onto a capillary of a sample diluted in water or in diluted BGE [9], was previously shown to be useful both in CZE and MECC [10]. During the stacking process, analytes present at low concentrations in a sample zone are concentrated into a short zone (stack). The detection of stacked analytes occurs in individual separated zones, which provide better separation efficiency and detection sensitivity.

A number of papers (presented in Table 1) dealing with CE applied to various types of inks have been published hitherto [11–26]. However, there is only one which reports successful result in the case of CE analysis of inkjet printing inks [11]. Therefore, the goal of this work was to make another attempt, taking what is best of CE and minimizing the problematic aspects. The MECC technique was considered useful for this purpose, because different inkjet printing inks samples can contain components with a wide variety of chemical behaviors and it can be very problematic and difficult to identify them by a single separation method. On the basis of publications about examination of other types of inks, the authors supposed that the utilization of micelles as a pseudostationary phase could facilitate the separation. It was essential from the forensic point of view to check if its discriminating power allows differentiation between similar inks, i.e. if the method can be useful in identifying forgery and evaluating the authenticity of a questioned inkjet printed document.

2. Experimental

2.1. Materials and samples

The chemicals used throughout the experiments were: sodium tetraborate decahydrate (POCH, Poland), sodium dodecyl sulphate(IV) (SDS) (Sigma–Aldrich, Germany), methanol (MeOH) (Merck, Germany), acetonitrile (ACN) (Merck, Germany), hydrochloric acid (Merck, Germany) and sodium hydroxide (Merck, Germany). All reagents and solvents were of HPLC grade or p.a. purity.

Printouts of pure inks of three colours: Yellow (C4838A), Magenta (C4837A) and Cyan (C4836A) were taken from the Business Inkjet 1200 Hewlett–Packard printer (the manufacturer of the most prevalent inks among Polish computer owners). Test pages of colour-mixed inks (in a rainbow style) were printed by printers listed in Table 2. All printouts were prepared using ordinary, commonly available printing paper – PolSpeed (International Paper, Poland) of 80 g m^{-2} basis weight and were stored in dry dark conditions at room temperature before use [27].

2.2. Preparation procedures

Stock solutions of 100 mM borate buffer and 200 mM SDS were prepared by weighing an adequate amount of sodium tetraborate and SDS, respectively, and dissolving in purified water. The two solutions and ACN were used for preparation of the background electrolyte (BGE). The BGE solutions were sonicated, filtered

Table 2
Printers with appropriate cartridges used throughout examinations.

No.	Company	Printer type	Cartridge index		
			Yellow	Cyan	Magenta
1	Hewlett–Packard	Deskjet D1360		HP 22 (C9352A)	
2		Officejet 5610		HP 22 (C9352A)	
3		PSC 1410		HP 22 (C9352A)	
4		Deskjet 895 Cxi		HP 23 (C1823)	
5		Deskjet 5150		HP 28 (C8728)	
6		Deskjet 3820		HP 78 (C6578)	
7		Deskjet 930 C		HP 78 (C6578)	
8		Deskjet 950 C/952C/959C		HP 78 (C6578)	
9		Deskjet F4280		HP 300xL (CC644EE)	
10		Photosmart C3100		HP 343 (C8766EE)	
11		Photosmart C4280		HP 351xL (CB338EE)	
12		Business Inkjet 1200	HP 11 (C4838A)	HP 11 (C4836A)	HP 11 (C4837A)
13	Canon	Pixma iP4500	CLI-8Y	CLI-8C	CLI-8M
14		Pixma MP210		CL-38	
15		Pixma iP1800		CL-38	
16		Pixma iP1900		CL-38	
17	Brother	DCP-135C	LC-970Y	LC-970C	LC-970M
18		MFC-215C	LC-900Y	LC-900C	LC-900M
19	Epson	Stylus D92	T0714	T0712	T0713
20		Stylus Photo R340	T0484	T0482/T0485	T0483/T0486
21	Lexmark	Lexmark X2530		35 Colour	
22		Lexmark Z615		26 Colour	

through 0.45 μm cellulose syringe filters Minisart RC15 (Sartorius, Germany) and stored in darkness until used. They were replaced after every five runs.

Before daily operation, the capillary was conditioned with MeOH for about 5 min, then with 1 M HCl for 5 min, 0.1 M NaOH for 10 min, water for 2 min, and finally, with the BGE for another 10 min. Between single runs, the capillary was rinsed with the same solvents but for a meaningfully shorter time (for 5, 2, 2, 1, and 1.5 min, respectively).

Pure inks (Magenta, Yellow, Cyan) and a mixture of them were extracted from paper in defined amounts of extracting agent (0.5 mL) in order to prepare stock solutions which could be used throughout optimization and validation stages. 100 disks (ø 0.4 cm) from printouts with pure colours or 12 squares (each of 0.9 cm × 1.2 cm) in tri-coloured rainbow style from specially printed test pages were cut out and extracted in 0.5 mL of extracting agents supported by 15 min of sonication. After that, the extracts were centrifuged (13,000 rpm for 5 min) in Eppendorf vials and transferred to new bottles in order to separate them from paper particles. 5 μL were taken from extracts prepared in such a way, diluted in 5 μL of the background electrolyte (injection solution) and then injected onto the CE instrument.

Real ink samples were collected in the form of 25 dots (ø 0.8 mm) of printed paper (0.13 cm^2 of surface area per 10 μL). Then, ink was extracted to 5 μL of BGE; the extraction was accelerated by sonication for 15 min at room temperature. After that, the supernatant was centrifuged (13,000 rpm for 5 min) to dispose of possible small pieces of paper and paper fibres, transferred to another micro-vial and diluted by 5 μL of the above mentioned injection solution. Samples prepared in such a way were ready to be directly injected into the CE instrument.

2.3. Instrumentation

A home-made syringe with a flat-point steel needle (0.8 mm ID) was used to punch out circles of printed paper in order to extract inks. The extraction process was conducted in a Sonic 3 ultrasonic bath (Polsonic, Poland). An MPW-250 centrifuge (MPW Med. Instruments, Poland) was used to centrifuge the samples before capillary electrophoresis analysis. The pH of BGEs was checked using a Microcomputer pH-meter CP-315 M (Elmetron, Poland) with combined electrode ERH-11 (Hydromet, Poland) calibrated on standard water buffers (POCh, Poland).

Electrophoretic measurements were carried out with the P/ACE MDQ Capillary Electrophoresis System (Beckman-Coulter, USA) using polyimide-coated fused silica capillary (75 μm ID, 60 cm total/50 cm effective length, Beckman-Coulter, USA). The injection was made by applying a pressure of 0.7 psi for 6 s. The separation process was conducted for 15 min with +30 kV applied. The electropherograms were recorded from 220 to 600 nm with the aid of a diode array detector (DAD). The capillary cassette and the sample storage module were maintained at constant temperature.

3. Results and discussion

3.1. Optimization of the method

3.1.1. Composition of background electrolyte

To determine the optimum composition of background electrolyte (BGE), 63 solutions containing borate buffer, SDS and ACN at different concentration levels within ranges 10–50 mM, 0–120 mM and 0–30% (v/v), respectively, were investigated. The pH values of these solutions ranged from 9.50 to 10.50. The aim was to select the composition providing the best analytical results in terms of the number, intensity and resolution of peaks in electrophoretic profiles. Cyan ink taken from a printout printed by a Business Inkjet 1200 (Hewlett–Packard) printer was analyzed.

BGE solutions consisting of just borate buffer proved to be a good choice for inkjet printing inks analysis. Components of inks gave rather well-developed and shaped peaks; however, some improvements in resolution and intensities of peaks were required. Addition of SDS improved resolution and intensity of peaks, increased their number and enhanced the stability of the baseline. Further improvement of resolution, intensity and shape of the peaks was caused by addition of ACN. It was ascertained that 10% of ACN allowed all peaks to be well separated and analysis to be completed in a satisfactory 15 min.

Table 3
Optimization of the composition of BGE solutions with regard to function *F* (see text).

l.p.	Borate buffer [mM]	SDS [mM]	ACN [%, v/v]	n	t_m [min]	RSD_{med}	F
1	40	10	10	5	9.0	0.3	9.3
2	40	20	10	8	10.0	0.5	12.1
3	40	30	10	7	12.4	0.5	8.2
4	40	40	10	8	12.3	0.5	10.8
5	80	40	10	10	19.8	0.6	8.1
6	80	50	10	12	18.5	1.2	6.7

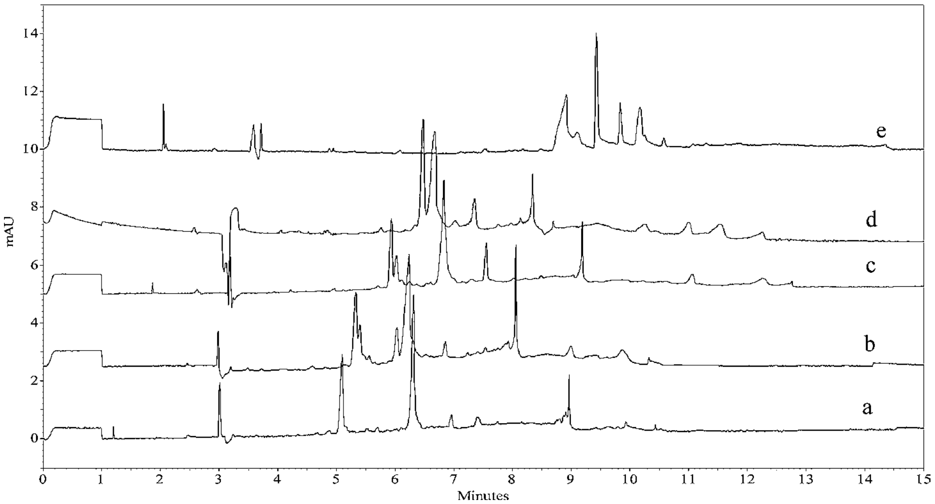

Fig. 1. Separation of components of Cyan ink extract (Business Inkjet 1200 printer) with the use of different BGE solutions containing borate buffer [mM], SDS [mM] and ACN [%] in the following concentration ratios: (a) 40/10/10, (b) 40/20/10, (c) 40/30/10, (d) 40/40/10, (e) 80/40/10.

For more careful optimization, five of the most promising three-component BGE solutions of different concentrations of borate buffer and SDS were used. This optimization was carried out on extract of Cyan ink from a printout created by a Business Inkjet 1200 (Hewlett–Packard) printer. As the selection criterion, the following function was proposed: $F = n^2/(t_m \cdot RSD_{med})$, where: n – the number of obtained peaks, t_m – migration time of the latest peak, RSD_{med} – median of relative standard deviations of migration times of peaks (of those whose area ≥1000 area units). The *F* values obtained for the examined BGE solutions are presented in Table 3 and the electropherograms of these solutions are shown in Fig. 1.

As seen, the BGE solution containing 40 mM sodium borate buffer, 20 mM SDS and 10% ACN offers a good compromise between resolution and analysis time and provides higher intensity of the important peak of blue dyes (see Fig. 1b at 8.0 min). Furthermore, in this case the concentrations of the components were great enough to reduce adsorption effects onto the capillary wall and minimize rinsing time between analysis runs.

There was one more advantage of using such a BGE solution: the differentiation of individual colours of Hewlett–Packard ink was possible with very good results. Every colour was typified by a electropherogram which is different from that of other colours, each having its own typical peak (for Yellow ink this was a peak at about 11.4 min, for Magenta ink at 6.5 and 7.6 min and for Cyan there were two characteristic peaks at 5.0 and 7.4 min).

On the basis of the obtained results, the authors ascertained that the BGE consisting of 40 mM borate buffer with 20 mM SDS and 10% (v/v) ACN was optimal in terms of resolution for the separation of inkjet printing inks extracted from paper. Thus, it was selected to be used in all further analysis.

3.1.2. *Instrumental conditions*

As the CE system produced by Beckman-Coulter allows adjustment of temperature of the capillary cartridge during performed analysis, the temperature was tested at three levels: 15, 25 and 35 °C. As temperature increased, the time of analysis was shorter and, consequently, the repeatability of measurements was improved (Table 4). However, it was noticed that at a temperature of 35 °C, no informative results were obtained. At this temperature, the Joule's heat was too high to be efficiently transferred outside the capillary and thus the heated BGE gave rise to current problems during the separation, leading to non-reproducible results.

One should be aware that in forensic examination, the volume of the sample solution should be minimal to avoid too great destruction of the examined document. According to the authors analytical experience, in the case of the Beckman-Coulter system, a volume not smaller than 10 μL was recommended to be used in order to ensure reproducible injections. This caused problems with too high vulnerability of a sample to evaporation and its adhesion to the wall of the vial. In addition, the ratio of water to organic solvent changed

Table 4
Migration time and repeatability of peaks ($n = 5$) obtained during MECC separation of Cyan ink extract (Business Inkjet 1200 printer) at 15 and 25 °C.

T [°C]	Parameter	Peak 1[a]	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7
15	$\bar{t}_m$ [min]	4.0	7.0	8.0	10.3	10.7	12.1	13.0
	RSD	0.2	0.5	0.5	0.4	0.6	1.3	0.5
25	$\bar{t}_m$ [min]	3.1	5.4	6.3	7.8	8.2	9.3	9.8
	RSD	0.0	0.3	0.4	0.5	0.4	0.3	0.3

[a] Peak 1 originating from EOF.

with time because of different evaporation rates of both phases. All of these factors may strongly influence analytical results.

Taking the above into account, the stability of samples was investigated. They were stored at three different temperatures in a sample storage module (10, 18, 25 °C) and at every temperature, analyses were performed 4 times after 0, 2, 6 and 12 h. In general, more significant changes of peaks in migration time were observed, the longer the sample storage time was. However, the temperature at which the sample solution revealed 6-h stability was 10 °C. When the sample storage module was maintained at 18 and 25 °C, considerable changes of migration time and intensity of peaks were visible after not more than 2 h. What is more, after 6 h almost the whole sample had evaporated and as a consequence the electropherograms were indecipherable.

As a result, all further studies of extracts of inkjet printing inks were conducted with the capillary cartridge maintained at 25 °C and the sample storage module at 10 °C. Under these conditions, the separation of inkjet printing inks could be achieved in less than 15 min.

3.1.3. *Optimization of sample preparation*

The most effective extracting agent and extraction parameters had been optimized previously [28]. The best extractant for isolation of inkjet printing inks from paper was a mixture of 40 mM borate buffer with 20 mM SDS and 10% acetonitrile, so nothing else but BGE was used in the separation process. Thus, the extracts based on BGE could be directly injected into the CE system without any special additional treatment. The remaining extracting conditions were found to be 15 min of sonication at room temperature and centrifugation (13,000 rpm) for 5 min.

The effect of the number of dots cut out of printed paper and taken for analysis was investigated. For this purpose, 10, 15, 20, 25 and 30 dots were taken, extracted and analyzed. As was expected, the intensity and number of registered peaks grew with the number of dots. In the case of 10 dots, only 4 peaks (excluding EOF peak) were visible, 15 dots gave 6 peaks, 20 dots – 7 peaks. Starting from 25 dots, 8 components of analyzed sample were extracted in sufficient amount to provide well-shaped and intensive peaks. A greater number of dots did not change the number of peaks; moreover, the intensity of peak 6 began to decrease.

One should remember that the number and intensity of the obtained peaks is closely connected not only with the number of original ink components but also with their chemical character and ability to bind irreversibly with the surface of the paper. What is more, in practice, the thickness of the ink surface can vary in different parts of documents or between printouts depending on several parameters, e.g. kind of ink, printer model and printing settings. Thus, to allow a comparison of all collected inks (Table 1), every extraction should be carried out with not less than 25 dots of printed paper: this number seems to result in not too much destruction of the analyzed document and at the same time is sufficient to obtain electrophoretic profiles with well-developed peaks of sufficient intensity suitable for reliable interpretation.

It was decided to exploit the normal stacking mode (NSM), based on hydrodynamic injection onto the capillary of a sample diluted in BGE [9]. Therefore, three BGE solutions of different dilutions were examined as injection solutions: pure BGE and BGE with water in ratios 1:9 and 1:99 (v/v). The results confirmed that stacking took place, especially, in the case of less intensive peaks – the observed increase was even up to 2.6 times. On the basis of obtained results, the BGE of the last dilution (1:99, v/v) was chosen for further examination.

3.2. *Validation of the method*

The validation study focused on the precision of the method in terms of repeatability and reproducibility of the migration time of peaks. The formula of inks is a trade secret and no reference samples were available; therefore, it was not possible to determine the accuracy.

3.2.1. *The repeatability*

In order to verify the repeatability, the following examinations were performed with the use of stock extracts of Cyan, Yellow and Magenta inks and their mixture (Business Inkjet 1200 printer): one sample prepared from the same portion of stock extract was analyzed 5 times (to estimate the influence of injection and instrumental parameters), and 5 portions of the stock solution of the particular extract were taken, prepared and analyzed independently of each other (to additionally estimate the influence of the sample preparation process). In addition, 5 samples of Cyan ink were independently taken for printouts, extracted, prepared (following the procedure of real sample preparation), and analyzed to estimate the precision of the whole method. The results obtained are presented in Table 5.

Table 5
The repeatability of migration time obtained for inks of separated colours and for a mixture of these inks (Business Inkjet 1200 printer).

Ink	Analysis	Parameter	Peak 1[a]	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7
Cyan	1 sample analyzed 5 times	$\bar{t}_\mathrm{m}$ [min]	3.1	5.3	6.3	8.1	8.4	10.4	–[b]
		RSD [%]	0.3	0.2	0.5	0.2	0.4	0.3	–
	5 samples independently prepared	$\bar{t}_\mathrm{m}$ [min]	3.1	5.4	6.3	8.1	8.4	10.4	–
		RSD [%]	0.4	0.6	0.7	0.2	0.3	0.7	–
	5 samples independently extracted	$\bar{t}_\mathrm{m}$ [min]	3.0	5.3	6.4	8.2	8.5	10.7	–
		RSD [%]	1.2	1.0	2.6	2.5	3.3	3.2	–
Yellow	1 sample analyzed 5 times	$\bar{t}_\mathrm{m}$ [min]	3.0	6.6	10.6	11.2	–	–	–
		RSD [%]	0.3	0.2	0.4	0.8	–	–	–
	5 samples independently prepared	$\bar{t}_\mathrm{m}$ [min]	3.0	6.7	10.8	11.3	–	–	–
		RSD [%]	0.5	1.1	1.0	0.8	–	–	–
Magenta	1 sample analyzed 5 times	$\bar{t}_\mathrm{m}$ [min]	3.0	6.4	7.0	7.9	11.0	–	–
		RSD [%]	0.16	0.2	0.3	0.3	0.3	–	–
	5 samples independently prepared	$\bar{t}_\mathrm{m}$ [min]	3.0	6.4	7.0	8.0	10.9	–	–
		RSD [%]	0.4	0.2	0.3	0.2	0.5	–	–
Mixture	1 sample analyzed 5 times	$\bar{t}_\mathrm{m}$ [min]	3.0	5.2	6.1	6.6	7.8	8.0	10.7
		RSD [%]	0.2	0.2	0.3	0.2	0.3	0.1	0.2
	5 samples independently prepared	$\bar{t}_\mathrm{m}$ [min]	3.1	5.4	6.5	6.9	8.2	8.8	11.5
		RSD [%]	0.2	0.2	0.4	0.4	0.1	0.1	0.3

[a] Peak 1 originating from EOF.
[b] Lack of peaks.

Table 6
The reproducibility of MECC separation for a mixture of inks depending on days, analysts, and capillaries (Business Inkjet 1200 printer).

Analysis	Parameter	Peak 1[a]	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7
5 days	$\bar{t}_m$ [min]	3.1	5.4	6.5	6.9	8.1	8.6	11.3
	RSD [%]	0.8	1.3	1.6	1.6	2.1	2.0	3.3
2 operators	$\bar{t}_m$ [min]	3.0	5.4	6.4	6.8	8.3	8.5	11.1
	RSD [%]	0.9	1.1	1.3	1.7	1.5	2.2	3.3
2 capillaries	$\bar{t}_m$ [min]	3.1	5.4	6.5	6.9	8.2	8.7	11.4
	RSD [%]	0.9	0.9	1.5	1.7	2.4	0.8	2.8

[a] Peak 1 originating from EOF.

The general observation was that the longer the migration time, the worse the repeatability of peaks. However, the method was found to be characterized by good precision (RSD < 3.3%). By looking at the RSD of the 5 repetitions of one sample, the conclusion was that the uncertainty coming from the apparatus had a limited impact on the precision. It seems that in the case of the sample preparation process with the use of the same stock extract, the sample injection and the instrumental parameters did not have a great impact on the precision of the method (in all cases RSD was less than 1.0%). On the other hand, the sampling conditions (printed areas selected to be punched) and extraction process were the most serious sources of errors of the obtained separation results.

3.2.2. The reproducibility

Reproducibility was examined with the use of a mixture of inks, with the samples being analyzed in the following way: (a) every day for 5 days (5 samples per day), (b) in one day by two analysts (5 samples per analyst), and (c) in one day using two different capillaries (5 samples per capillary). The results are presented in Table 6.

As seen, the change of capillaries affects reproducibility slightly less than the change of days and analysts. However, the RSD values were not significantly greater than 3.3% regardless of the conditions in which the analyses were performed. This confirmed that the precision of the method depended mainly on the preparation of the sample at the stage of selection and cutting out of printed paper pieces and their extraction.

As can be seen from Table 6, the EOF rate is almost constant during all 25 runs, showing a relative standard deviation of 0.8%. On the other hand, the migration time of the other components of inks increases slowly against number of runs. This was suspected to be a consequence of the relatively great change in the BGE composition (evaporation of ACN, electrolytic degradation). Therefore, the authors recommended using fresh BGE after every 5 runs.

Additionally, the dependence of the age of the BGE on the separation process was tested. Namely, three BGE solutions: 6-month-old, 3-month-old and freshly prepared (i.e. after 3 days of reaching of micelle equilibrium) were used for analysis. The effect was distinguishable: comparing first and last ones, the shift was ca. 0.2 min for the EOF peak and 1.8 min for last peak. That is why, in order to be reliable, the comparative analysis should be performed with the use of the same BGE, which is not older than a few weeks.

As the final conclusion of the validation study, it was ascertained that the method is capable of offering satisfactory results in terms of precision provided that a sample is analyzed with the use of as fresh BGE solution as possible, and preferably by the same operator using the same capillary. In any case, special attention should be paid to careful preparation of the sample at the sampling and extraction step.

3.3. Application of the method

To demonstrate and confirm the applicability of the developed method, samples of mixed inks taken from printouts were analyzed. A library of electrophoretic profiles was created for all the collected printouts made by printers of different models and producers (Hewlett–Packard, Epson, Brother, Lexmark and Canon; for details see Table 2).

In Fig. 2, the results for a sample taken from Business Inkjet 1200 printer (Hewlett–Packard) are shown. Although small changes in migration times were observed in comparison with those obtained for pure inks, peaks of ink components can be found on the electropherogram of sufficient intensity to be distinguished, when compared with known spectra taken from the library (with the exception of Cyan dyes, which do not give bands within 200–600 nm). As spectra related to both Magenta and Yellow colours were undoubtedly identified, the corresponding peaks can be classified as coming from Magenta and Yellow inks.

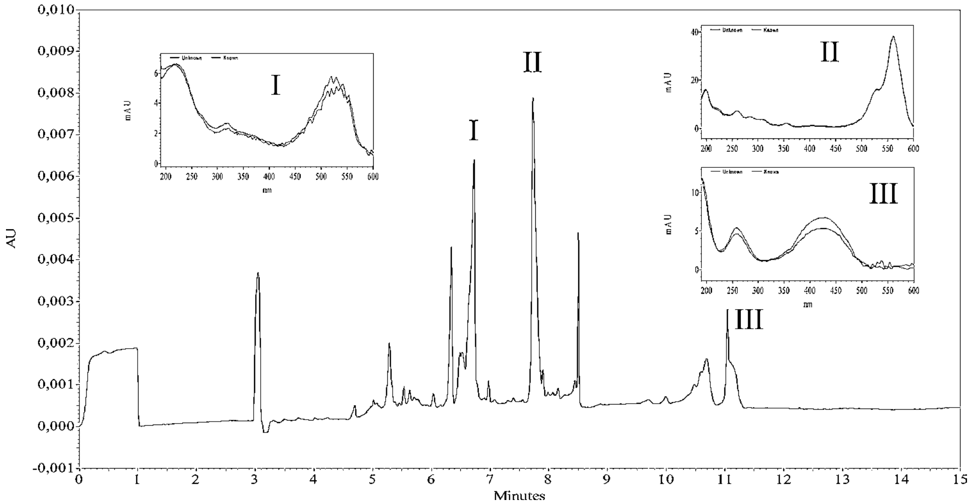

Fig. 2. Electropherogram of a mixture of inks in unknown proportions (Business Inkjet 1200 printer): I, II, III – spectra of unknown peaks compared with spectra of Magenta (6.5, 7.6 min) and Yellow (10.8 min) inks taken from spectra library.

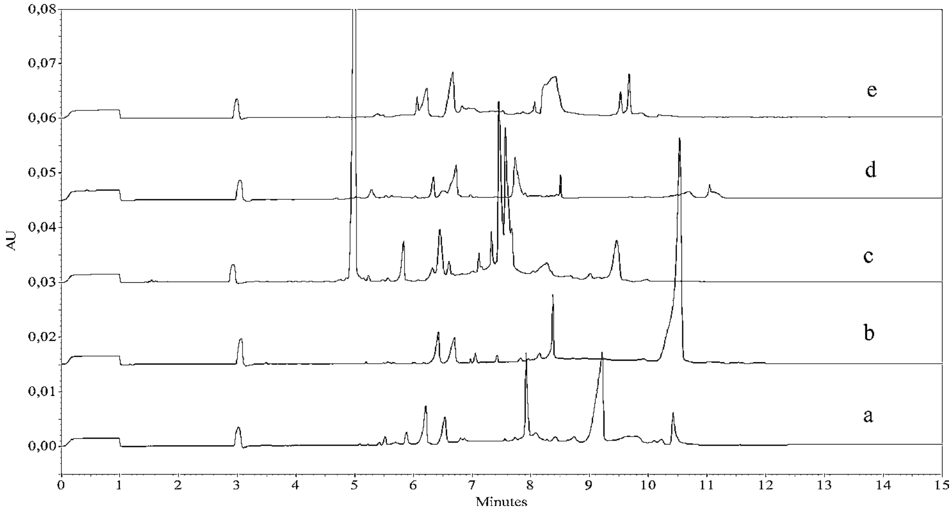

Fig. 3. Electropherograms of mixed-inks from printers of different producers: (a) Brother (DCP-135C), (b) Canon (Pixma MP210), (c) Epson (Stylus Photo R340), (d) Hewlett–Packard (Business Inkjet 1200), (e) Lexmark (2530×); peaks originating from paper are marked with asterisks.

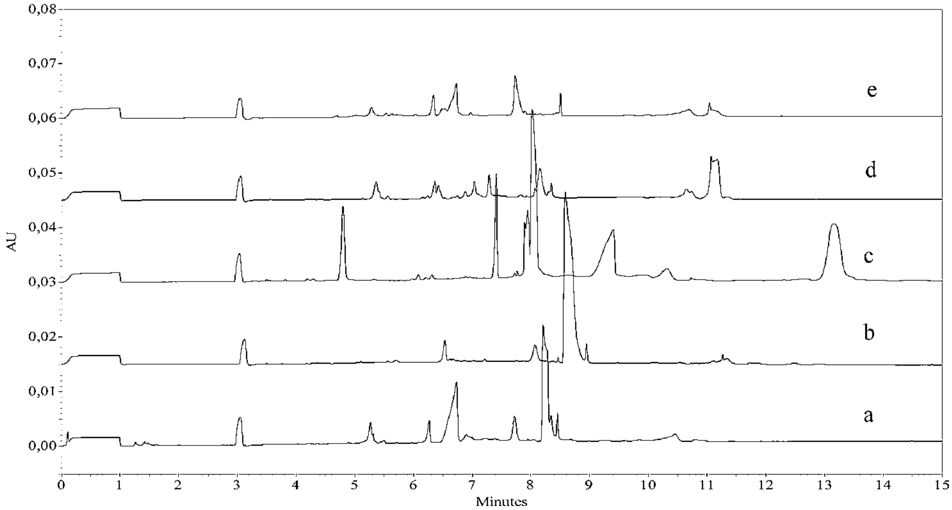

Fig. 4. Electropherograms of mixed-inks from four different models of printers produced by Hewlett–Packard: (a) PSC 1410, (b) Photosmart C3100, (c) Deskjet F4280, (d) Deskjet 895 Cxi, (e) Business Inkjet 1200; peaks originating from paper are marked with asterisks.

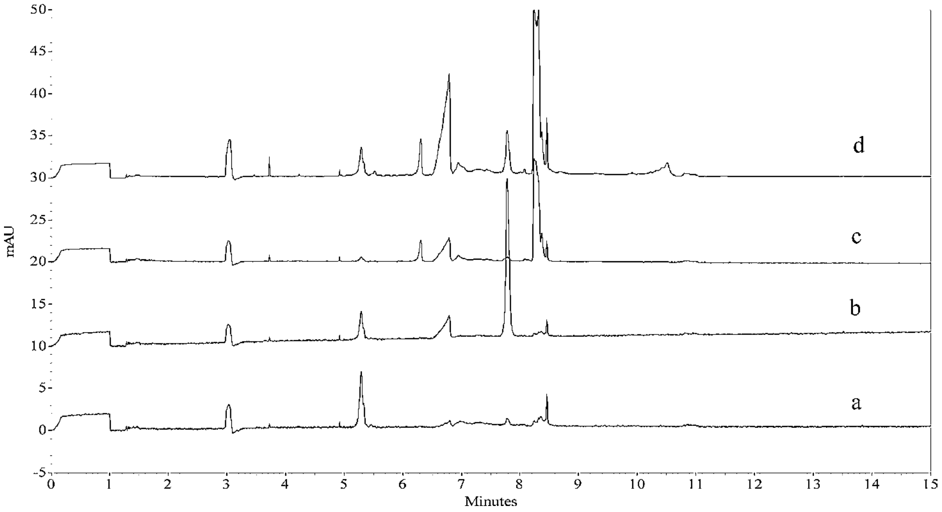

Fig. 5. Multielectropherogram of mixed-ink from PSC 1410 printer. Electropherograms at: (a) 600, (b) 570, (c) 370, (d) 220 nm; peaks originating from paper are marked with asterisks.

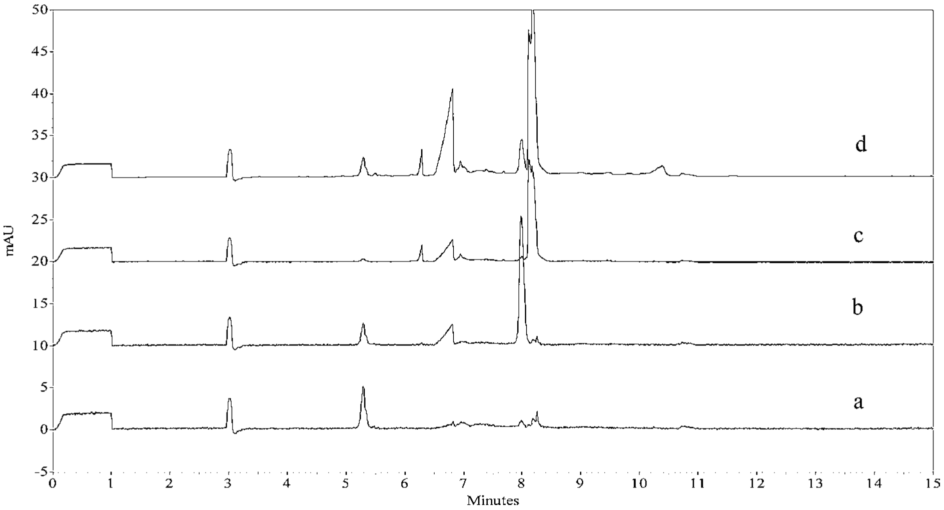

Fig. 6. Multielectropherogram of mixed-ink from DeskJet 930C printer. Electropherograms at: (a) 600, (b) 570, (c) 370, (d) 220 nm; peaks originating from paper are marked with asterisks.

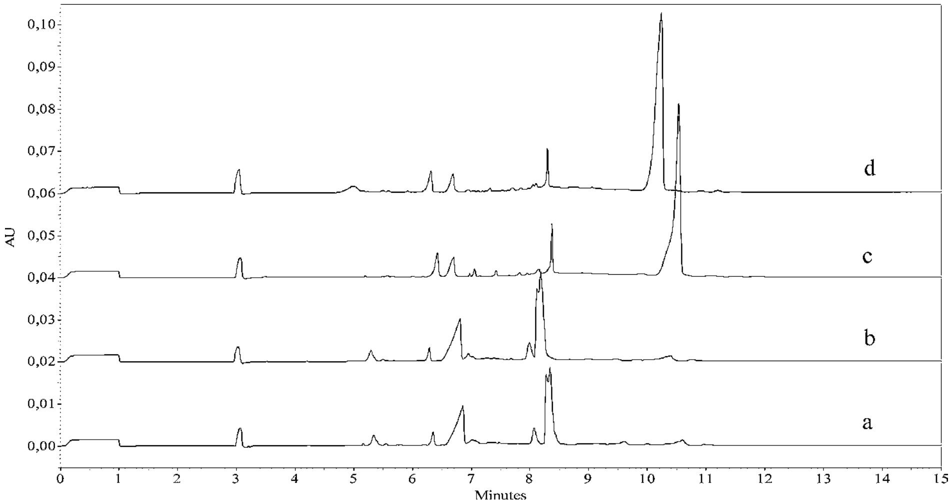

Fig. 7. Electropherograms of mixed-inks from two different models of printers produced by Hewlett–Packard (equipped with cartridge HP-78): (a) Deskjet 3820, (b) Deskjet 930C and produced by Canon (equipped with cartridge CL-38): (c) Pixma MP210, (d) Pixma iP1900; peaks originating from paper are marked with asterisks.

Fig. 3 presents a comparison of electropherograms of inks produced by various manufacturers: Brother, Canon, Epson, Hewlett–Packard and Lexmark. As expected, no difficulties were encountered in differentiating between inks of different producers. Inks differ either generally, i.e. exhibiting completely different electrophoretic profiles, or to a lesser extent, i.e. either in the migration time of a major peak (e.g. intensive peak at 5 min for Epson ink, a lone peak at 10.5 min for Brother ink) or in lack of peaks in particular regions. On the other hand, the similarity of inks was visible in some part of the electropherograms (e.g. peaks at about 6.5 min corresponding to inks of Brother, Epson and Lexmark printers presumably originate from the same chemical class of yellow dyes). However, it can be generalized that the method is a useful tool in discriminating between inkjet inks taken from printers of different producers.

Results of the comparative analysis of inks originating from selected models made by the same producer (Hewlett–Packard) are shown in Fig. 4. As can be seen, the differences between chemical compositions of these inks are also great enough for them to be disclosed using the proposed method. The electropherograms revealed that the analyzed inks had similar main colorants (e.g. peaks at about 6.5 min, electrophoretic profiles a and e) but, in general, they had been produced on the basis of different dyes and additives (e.g. peak at 4.8 min, electrophoretic profile c).

However, it is also possible that inks taken from different printers made by the same producer consist of similar or even the same dyes and, consequently, produce very similar electropherograms. As an example, the electrophoretic profiles obtained at 220 nm for inks from models PSC 1410 and Deskjet 930C (Hewlett–Packard) are presented in Figs. 5 and 6 (electropherograms d). In such cases, it is recommended to compare a set of electrophoretic profiles obtained at different wavelengths, as such multielectropherograms are capable of displaying more peaks or some changes in the peak intensities. For instance, the peaks corresponding to Cyan ink (8.5 min) are more and more visible at 370, 570 and 600 nm for the PSC 1410 printer, but not for the Deskjet 930C printer. What is more, the Magenta peaks (at 7.8 min in Fig. 5 and 8.0 min in Fig. 6) revealed not only differences in migration time, but also, in electrophoretic profiles b, discrepancies in intensity. Thus, although differentiation of inks from these two models of printers was difficult, it was feasible.

The most difficult problem with comparative analysis of electropherograms occurs when the same cartridge is used by two different printers. For example, in Fig. 7, the electrophoretic profiles

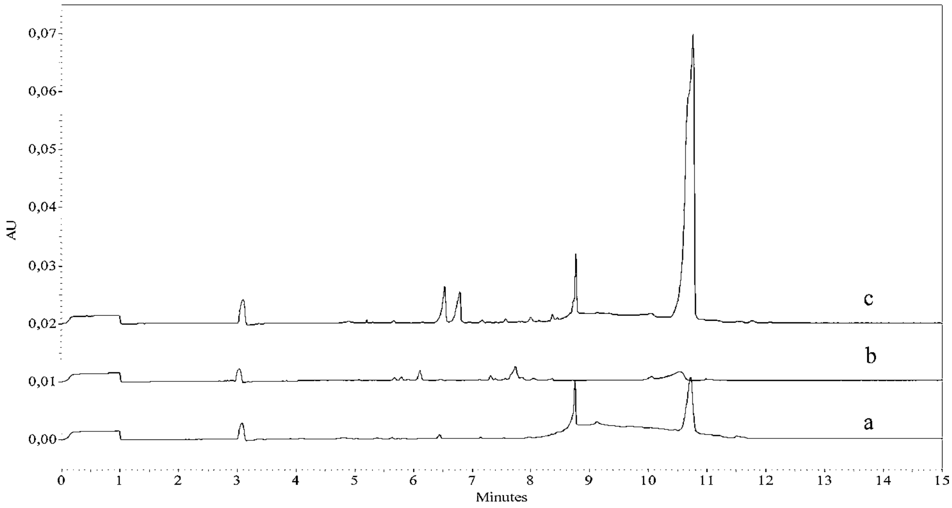

Fig. 8. Electropherograms of ink extracts taken from: (a) a bus ticket of unknown origin, (b) an original bus ticket used in Krakow in Poland, (c) mixed-inks from Pixma iP4500; peaks originating from paper are marked with asterisks.

of inks coming from two Hewlett–Packard printers equipped with an HP-78 cartridge and from two Canon printers working with a CL-38 cartridge are presented. The similarity of the electropherograms is so great that there is no basis to any claim about the different origin of the inks. Even if the electrophoretic profiles at different wavelengths were scrutinized there were no sufficient differences enabled the authors to change such a conclusion.

On the other hand, the database with electrophoretic profiles of inks from particular types of cartridge is very useful in group identification of inkjet printing inks and in comparing them with other kinds of inks (e.g. stamps inks) or ones used in printing for special purposes such as public utility (e.g. tickets, postage stamps or cheques). Fig. 8 presents an example result of an intralaboratory test, which was carried out in order to confirm the possibilities of the optimized method in this field.

A fake ticket, printed on an unknown printer, was a very good copy of the original: both were a blue monochromatic colour. Both tickets were analyzed and the electropherograms obtained (Fig. 8) revealed that one was indeed a forgery (compare electrophoretic profiles b and c). It was also detected that the fake ticket was probably printed using a Canon printer – model Pixma iP4500 (compare electrophoretic profiles a and c). The difference between the electropherogram of the unknown ink extract and the one taken from the previously created library of electrophoretic profiles (see region from 6 to 8 min) was caused by the blue colour of the ticket: during the printing process, the blue hue is obtained by mixing Cyan with an admixture of Magenta. Therefore, peaks originating only from these inks were observable on the electropherogram of the fake ticket (on the contrary to electropherogram from library where peaks of three colours of inks were visible). For the same reason, the intensity of the peak coming from the supplementary Magenta ink (at 10.8 nm) was lower.

4. Conclusions

On the basis of the performed investigations and results, the authors were willing to state that the ideal separation was obtained with a BGE composed of 40 mM sodium borate with 20 mM SDS and 10% (v/v) of ACN, and fused silica capillaries with an inner diameter of 75 μm with a total length of 60 cm. The best parameters for carrying out reliable analysis and comparison of electropherograms were found to be: temperature of capillary – 25 °C, temperature of storage of the sample – 10 °C, injection solution – BGE with water (1:99, v/v) and amount of sample – 25 dots. Under the above-mentioned conditions, analyses were shown to be highly reproducible from run-to-run and only a little less from day-to-day.

It was shown on the basis of analyses of 22 inkjet printing inks from 5 different manufacturers that it is possible to distinguish most of them from each other. Even if the difference between electropherograms is only slightly visible, better differentiation is possible using multielectropherograms. In addition, the identification of main components is also possible by comparison of recorded UV–vis spectra. This procedure can also be applied to discrimination and group identification of ink components, and thus facilitates the examination of documents' authenticity or makes it more efficient. What is more, an important advantage of the proposed method is the possibility of using a relatively small sample for analysis. This is an important factor when dealing with a document of high evidential value and thus when sampling often needs to be reduced to a minimum.

To summarise, the technique has already proved to be effective. Nevertheless, additional examinations are planned, encompassing a wider sample set of inks originating from different manufacturers. This will enable us to evaluate the usefulness of the technique for routine forensic analysis and to create a searchable library (database) of electrophoretic profiles of the numerous inks available in Poland. Moreover, due to the high standards applied in ink production processes the differences between batches are very subtle and a method with great discriminating possibilities needs to be developed. Application of a method with higher discriminating power – both qualitative and quantitative – is planned for problematic comparisons of not only a brand but also a batch of inks. Supported by internal standards, this method will enable us to conduct comparative analysis based on the ratio of peaks' integrated areas.

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