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Application of CE-MS to examination of black inkjet printing inks for forensic purposes

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ABSTRACT

The potential of capillary electrophoresis coupled to mass spectrometry with electrospray ion source and time of flight analyser (CE-ESI-TOF-MS) in the analysis of inkjet inks was investigated. The developed and validated method allowed reliable and repeatable analysis of black inkjet inks extracted from printouts. Over a dozen inkjet printouts printed on various printer models from different manufacturers were analysed under selected conditions to determine the variation of chemical composition of inks between different brands and types. It was ascertained that the developed method is capable of revealing qualitative differences between ink samples. For most of the investigated inks, the studies showed the presence of a characteristic mass spectrum originating from the surfactant or polymer. The mass distribution of the additive is distinctive for some inkjet ink producers, and allows for group identification of inks. The results showed the strength of the CE-ESI-TOF-MS method as an effective technique for forensic purposes, requiring a small amount of inkjet ink samples and giving analytical information that is useful in the identification of compounds.

1. Introduction

Questioned documents produced on modern office equipment are a frequent problem in today's world. A large share of forgeries or counterfeits relates to inkjet printed documents, and in particular, to the most widely used black inkjet printouts. The detection of this type of crime is becoming increasingly difficult. The reason for this phenomenon is primarily on-going advances in digital printing. The fundamental step in verifying the authenticity of documents is still chemical analysis of inks, and in the case of inkjet inks, especially black inkjet inks, this is still a challenge for forensic document examiners. In particular, the method allowing well-defined chemical composition of a black inkjet ink to be attributed to definite type of printers, or even better to individual printer, would be especially desirable in real case forensic work.

Inkjet technology requires a specific composition of inks, different from that found in writing inks. Most inkjet inks in desktop printers are highly-engineered, multicomponent solutions of colourants (dyes and/or pigments, below 10% *w/w*) and various additives in solvent (50–90% *w/w*). Inkjet inks can be water-based, solvent-based or UV curable. However, in common desktop inkjet technology, drop on demand, water-based inkjet inks are the most frequently utilized. The additives provide the appropriate physicochemical properties (such as surface tension, conductivity, viscosity, shelf life, and drying time), in order to meet demanding performance specifications by inks. Among the additives used in inkjet inks are polymeric binders, co-solvents (e.g. polyethylene glycol), dye-solubilizing agents (e.g. 2-pyrrolidone), surfactants (e.g. silicon or fluorosurfactants), pH buffers, electrolytes, defoamers, and biocides [1,2]. The main component of black inkjet inks is carbon black pigment, because of its desirable image performance, high colour strength and optical characteristics [2]. On the other hand, colour inkjet inks are usually dye-based.

The examination of inkjet inks on questioned documents is, at first, confined to non-destructive analysis, with the use of microscope, video spectral comparator (VSC) or micro-spectrometry [3] and Raman spectrometry (RS) [4] techniques. However, the effectiveness of non-destructive techniques is limited in the analysis of such images or texts (composed of separated small dots of different ink colours, very often superimposed on each other), and, in further stages, destructive analysis is usually employed. Standardized methodology for destructive ink analysis is based on the screening of ink sample dye composition. Sophisticated separation techniques like thin layer chromatography (TLC) [5], high performance liquid chromatography (HPLC) [5,6] or capillary electrophoresis (CE) [7–10] are most widely used for this purpose. Modern mass spectrometry techniques (MS), including laser desorption mass spectrometry (LDMS) [4,11,12], fast atom bombardment mass spectrometry

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(FABMS) [11], time of flight secondary ion mass spectrometry (TOF-SIMS) [13] or direct analysis in real time ion source mass spectrometry (DART™-MS) [14], are increasingly applied to the examination of inkjet inks.

Most examinations have involved colour inkjet inks. Mazzella [3] collected visible spectra of pure magenta and cyan inks (original and substituted). The results showed that the effectiveness of non-destructive micro-spectrometry is limited to the comparative analysis of inkjet inks on the same paper sheet. TLC and HPLC techniques were used by Poon et al. [5] for analysis of colour inkjet inks (both original and substituted inks) taken directly from cartridges and/or extracted from ink print on paper (3 mm × 10 mm). The examined inks of different producers were easily differentiated from each other. However, the analysis of printouts required a large sample size, and the authors were not successful in the analysis of inks of the same producer. In another study, Lofgren et al. [6] were able to differentiate between inks from different production lots from the same manufacturer using the HPLC technique. Another separation technique, micellar electrokinetic capillary chromatography with diode array detection (MECC-DAD), was also applied to the analysis of colour inkjet inks. Xu et al. [7] optimized the MECC-DAD method, making it suitable for separate compounds with very similar structures and their derivatives, which are encountered in writing and printing inks. Szafarska et al. developed the MECC-DAD method of analysis of colour inkjet inks extracted from paper [8] and applied it for forensic purposes [9]. Based on electrophoretic profiles obtained in the UV–vis range, both differentiation and group identification of inkjet inks were possible. In turn, MS techniques like FABMS and LDMS were applied by Grim et al. [11] to the direct analysis of inks on paper. When using the LDMS technique, only dyes were detected, in contrast to the FABMS technique; however, LDMS was the preferred method for analysis of ink components on paper, due to its flexibility and simplicity.

There are only a few reports concerning analysis of black inkjet inks for forensic purposes [4,10,12,14]. The MECC-DAD method [10], previously used for analysis of colour inkjet inks, was also successful in the examination of black inkjet inks. However, some difficulties with extraction from paper or differentiation of inks of other producers were encountered. Donnelly et al. [12] revealed the presence of molecules that contain different numbers of C_2H_4O units, most likely derived from polyethylene glycol or surfactant, in black inkjet inks by the LDMS technique. A comprehensive approach (by means of RS and LDMS) was applied by Heudt et al. [4] to the study of both colour and black inkjet inks. The RS technique allowed differentiation of colour inks, but it was insufficient in analysis of black inkjet printouts. The spectra confirmed the presence of copper phthalocyanine derivatives in cyan inks and carbon black (or modified carbon black) in the investigated black inks. The LDMS technique showed polymers with mass distribution and end groups characteristic for each manufacturer of inkjet inks. Distinguishing between inks from the same producer by the LDMS method was also possible to some extent. Houlgrave et al. [14] applied the very sensitive DART™-MS technique to the analysis of inkjet inks, tested as separated inks taken from ink cartridges, inks printed on paper, and inks extracted from paper. Characteristic mass spectra of black inks were qualitatively reproducible. The authors created a library based on various combinations of cyan, magenta, yellow, and black inks, supporting analysis of questioned inkjet printouts.

The only studies using the combination of the separation efficiency of the CE technique and the high sensitivity and resolution power of the MS technique for the analysis of colourants relate to the examinations of natural dyes [15], dyes extracted from fibres [16], food dyes [17,18], and ecotoxic dyes [19–21]. For most of the aforementioned applications, capillary zone electrophoresis (CZE) with background electrolytes (BGE) composed of ammonium acetate in a mixture of acetonitrile (ACN) and water in different pHs was used. The MECC technique in its basic form cannot be used in combination with MS detection due to the surfactant present in the BGE, impeding the ionisation process [17]. MS systems with electrospray (ESI) interface for ionisation, and different analysers (ion trap, quadrupole and micro-time of flight) were used in the experiments. The list of CE-MS studies is not comprehensive, but shows that CE-MS is an important tool in modern analytical chemistry.

The possibility of comparative analysis of inks from different manufacturers was demonstrated; however, the differentiation of inks within a given producer is still challenging. Hence the need to develop a modern, effective and reliable method for the differentiation and identification of black inkjet inks from questioned documents is evident. The CE method with MS detection is expected to be able to minimize the amount of sample required for analysis and to significantly improve qualitative ability in comparison to use of a DAD detector. It seems to be appropriate for the analysis of samples where many unknown compounds can be present. To our best knowledge, CE-MS has not yet been used in forensic studies of inkjet inks and the potential of this technique in the analysis of inkjet inks still needs to be investigated.

The aim of this paper was to develop a CE-ESI-TOF-MS method and to verify the reliability of this analytical tool in the examination of inkjet ink components extracted from black printouts for forensic purposes. Over a dozen inkjet printouts made with the use of original inks of different brands and types were analysed. The possibility of identifying unknown compounds was also studied.

2. Material and methods

2.1. Capillary electrophoresis

A commercial CE system, PA 800 plus Pharmaceutical Analysis (Beckman Coulter, USA) equipped with Karat 32 software (Beckman Coulter, USA), was used. Separations were performed using a fused-silica capillary with a length of 100 cm, an internal diameter (i.d.) of 50 μm and an outer diameter (o.d.) of 375 μm (Beckman Coulter, USA). Polyimide coating was removed from the capillary ends to avoid polyimide swelling. The new capillary was rinsed with methanol for 10 min, followed by 1 mol/l HCl for 5 min, water for 3 min, 0.1 mol/l NaOH for 30 min, water again for 3 min and BGE for 30 min, at 25 psi at each stage. Pre-sequence conditioning and post-sequence rinsing were shortened processes of the new capillary treatment, with BGE exchanged for air in the case of post-sequence rinsing (for 5, 7, 3, 14, 3, and 21 min, and 3, 2, 3, 3, 4, and 4 min, respectively). Between runs, the capillary was flushed with methanol for 3.5 min, water for 2.5 min, 1 mol/l NaOH for 0.6 min (at 2 psi), water again for 4.5 min, and BGE for 5 min. The BGEs were replaced after every four runs. The samples were injected into the anodic end of the capillary in hydrodynamic mode at 0.7 psi for 7 s (0.4 and 1.0 psi as well as 4 and 10 s were tested at the method development stage). Separations were performed at 25 °C with an applied constant voltage of 30 kV. Samples were stored at 10 °C (18 and 25 °C were also tested). The pH* (apparent pH) values of the BGEs were measured by a pH 500 Benchtop Metre (Beckman Coulter, USA) with a gel-filled pH electrode with built-in automatic temperature compensation (Beckman Coulter, USA). The prepared BGEs were filtered through a 0.45 μm syringe filter (Cronus, UK) and centrifuged in an Allegra X-30R Centrifuge (Beckman Coulter, USA) to remove air bubbles.

2.2. Mass spectrometry

A micrOTOF II (Bruker Daltonics, Germany) TOF spectrometer, fitted with an ESI source, and operated in the positive ion mode was coupled to the CE system. Bruker Compass software (Bruker Daltonics, Germany) was used for MS control, data acquisition and processing. Compass Isotope Pattern (Bruker Daltonics, Germany) was used to calculate the theoretical *m/z* ratios. In order to couple CE with MS, an Agilent CE-ESI-MS sprayer (Agilent Technologies, USA) was applied. The fused-silica capillary protruded approximately 0.1 mm out of the sprayer tip. The ESI-TOF-MS spectrometer was run at 4.5 kV, and the pressure of the nebulization gas (N_2) was set at 0.4 bar. The drying gas (N_2) flow rate was 4.0 l/min and the source temperature was set at 180 °C. The mass spectrometer was scanned from *m/z* 50 to 1000 in full scan mode. The MS transfer parameters, such as the voltage at capillary exit (90 V), skimmer (30 V) and hexapole (190 Vpp), or time of transfer lens (32 μs) and pre-pulse storage lens (14 μs), were optimized for maximum sensitivity of analytes. A sheath-liquid solution (0.2% *v/v* formic acid in isopropyl alcohol:water, 1:1 *v/v*) was delivered by an automatic syringe pump (KD Scientific, USA) at a constant flow rate of 3 μl/min. The sheath-liquid solution was degassed for 10 min in an ultrasonic bath (Polsonic, Poland) prior to use. ESI-TOF-MS calibration was performed at the end of each run in the positive ion mode, using sodium formate clusters $Na(NaCOOH)_n$ for *n* from 2 to 11. Calibration solution was produced at the outlet of the capillary by mixing 0.5 mol/l NaOH with sheath-liquid.

2.3. Chemicals and samples

Acetonitrile (ACN, ≥99.9%), acetic acid (≥99.8%), ammonium acetate (≥99.0%), methanol (≥99.9%) and isopropyl alcohol (≥99.9%) were obtained from Sigma-Aldrich (Germany). Formic acid (>99.9%), hydrochloric acid (32%, extra pure) and dimethyl sulphoxide (DMSO, >99.9%) were purchased from Merck (Germany). Sodium hydroxide (30%, extra pure) and pyridine (≥99.5%) were obtained from POCH (Poland). The following dyes supplied by Sigma-Aldrich (Germany) were used: Methyl Violet (MV), Patent Blue (PB), Rhodamine B Base (RBB), Victoria Blue B (VBB), and Victoria Blue R (VBR). The dyes were of analytical grade and were used without further purification. Ultra-pure water (18 MΩ cm), from a Milli-Q Integral 3 purification system (Merck Millipore, USA), was used in all procedures.

Stock solutions of dye standards at a concentration of 3 mg/ml were prepared by dissolving accurately weighed amounts of pure dyestuffs in methanol. The prepared solutions were stored in dark glass bottles at 4 °C. 0.1 mol/l stock solution of ammonium acetate was prepared by weighing pure ammonium acetate and diluting with water. BGEs containing acetic acid, ammonium acetate, acetonitrile and water in the following proportions: 0.01–0.1% *v/v*, 5–50 mmol/l, 5–50% *v/v*, and 50–95% *v/v*, respectively, were prepared. The sheath-liquid was prepared by adding formic acid to a mixture of isopropyl alcohol:water, 1:1 *v/v*. All BGEs and sheath-liquid were stored in dark glass bottles, at room temperature.

13 black inkjet printouts were printed on Polspeed white office paper (International Paper, Poland), in the form of 33 rectangles (9 mm × 12 mm), by 13 different models of inkjet printers made by the five most common manufacturers in Poland: Hewlett-Packard, Brother, Canon, Lexmark, and Epson. All printers were equipped with original colour and black inkjet inks. A complete list of examined inkjet ink samples is presented in Table 1. Ink samples were allowed to dry before examination. A4 sheets of five different commercially available standard white office papers made by two different producers: Polspeed (80 g/m^2), Pollux (80 g/m^2), Poljet (90 g/m^2) and Rey (80 g/m^2) produced by International Paper (Poland), and Presentation (100 g/m^2) by Navigator (USA), were investigated. The printouts were stored in plastic bags in the dark at room temperature.

Table 1
List of inkjet inks examined in the study.

Order no.	Manufacturer	Model	Black cartridge number	Colour cartridge number
I	Hewlett-Packard	Business Inkjet 1200	HP10	HP11
II	Hewlett-Packard	Deskjet 4280F	HP300	HP300
III	Hewlett-Packard	Deskjet 5550	HP56	HP57
IV	Hewlett-Packard	Photosmart B109a	HP364	HP364
V	Hewlett-Packard	Deskjet 5652	HP56	HP57
VI	Brother	DCP 135C	LC970B	LC970(C, M, Y)
VII	Brother	MFC 5440	LC900B	LC900(C, M, Y)
VIII	Canon	I 965	BCI6	BCI6
IX	Canon	MP 240	PG510	CL511
X	Canon	IP 1900	PG37	CL38
XI	Canon	MP 210	PG37	CL38
XII	Lexmark	2530X	34	35
XIII	Epson	92D	T0711	T07(12–14)

2.4. Sample preparation

A mixture of equal volumes of stock solutions of five dye standards: MV, PB, RBB, VBB, and VBR was prepared. The examined dye samples were obtained by taking 1 μl of the mixture of dyes, evaporating methanol at 60 °C in a stream of N_2 using an evaporator (Liebisch Labortechnik, Germany) and adding 200 μl of appropriate solution.

A homemade flat needle with 0.63 mm i.d. was used to cut 10 chads (or 5, 15, 20 and 25 at the method development stage) from the inkjet printouts. The chads were placed into glass vials. One out of four extractants (DMSO, methanol, a mixture of pyridine: water, 1:1 *v/v*, or a mixture of acetonitrile:water, 1:3 *v/v*, containing 5 mmol/l ammonium acetate and 0.01% *v/v* acetic acid) was used in amount of 10 μl (or 5 μl at the method development stage) in the extraction process. Extraction was carried out in an ultrasonic bath at room temperature for 15 min. The supernatant was transferred to an insert using a micro-syringe and the extractant was evaporated to dryness under a stream of N_2 at 60 °C using an evaporator. The residue was dissolved in 20 μl of injection solution and centrifuged at 14,800 rpm for 5 min using a Microfuge 16 Centrifuge (Beckman Coulter, USA) prior to CE analysis. A paper blank was obtained by collecting 10 chads of blank paper near the sampling location of the ink. The blank was extracted and analysed in the same manner as the ink sample.

3. Results and discussion

3.1. Separation conditions

Five standard dyes (with theoretical *m/z* values for $[M^+]$ or $[M+H]^+$): MV (*m/z*=358.2278), PB (*m/z*=545.1775), RBB (*m/z*=443.2329), VBB (*m/z*=470.2591), and VBR (*m/z*=422.2591) were selected for developing the method. For the CE, such conditions as compositions of BGE and sample solution, and sample storage temperature were optimized. The analysis encompassed the following: detection of the maximum number of components, symmetrical peak shapes, sufficient resolution of the relevant peaks and acceptable total analysis time. Preliminary experiments allowed a mixture of acetic acid, ammonium acetate, ACN, and water to be selected as the BGE. A multivariate procedure

was used to estimate the main interactions of individual components of the BGEs. Three out of four BGE components (water was an adjusting component) were tested at two levels of concentration: 0.01 and 0.1% *v/v* for acetic acid, 5 and 50 mmol/l for ammonium acetate, 5 and 25% *v/v* for ACN. The levels were set based on information from the literature [15,16,18–21]. The pH* of the tested BGEs varied in the range from 4.23 to 6.34. Selection of the best BGE composition was on the basis of three responses: L_R, which is the number of resolved peaks with height exceeding 3000 arb. unit; *t*, which is the migration time of the last migrating dye in each measurement, and the *CV* value for the time of the last migrating dye from three replicates for each BGE. The following function (*F*) was chosen as the primary criterion of selection of the BGE composition: $F=L_R^2/(tCV)$. A list of the tested BGEs, values of responses for each BGE and *F* values are presented in Table 2.

As an example, extracted ion electropherograms from CE-ESI-TOF-MS analyses of dye mixtures for BGEs 1, 6 and 7 are shown in Fig. 1. For each BGE composition, 5 peaks from 5 analysed dyes were observed. Well-separated peaks were obtained for the BGEs containing ammonium acetate at a concentration of 50 mmol/l and 25% *v/v* ACN (BGEs 1 and 3 in Table 2) in contrast to e.g. BGE 6 (compare Fig. 1A and B for BGEs 1 and 6, respectively). In each run, the slowest migrating component was the acid dye PB. BGE composed of ammonium acetate and acetic acid at low levels and ACN at a high level allowed the separation to be completed in less than 11 min (see Fig. 1C).

On the basis of *F*-values, BGE 7, composed of the 1:3 *v/v* mixture of ACN and water containing 5 mmol/l ammonium acetate and 0.01% *v/v* acetic acid, exhibited the most effective influence on separation of dyes. It offered a good compromise between resolution and analysis time and provided higher intensity of peaks of dyes. Due to the specificity of the MS detector, allowing independent detection of analytes with different masses, worse separation of sample components is less important than long analysis time. Short analysis time is particularly important because of the possibility of occurrence in inkjet inks of acid dyes migrating more slowly than PB. Additional studies showed that increasing the ACN content, resulting in improvement in separation conditions, caused unwanted modifications in the capillary. It was found that using BGEs with ACN content in the range 50–100% *v/v* for an extended period of time (about 10 days) caused irreversible changes in the inner wall of the capillary and degradation of the polyimide coating.

Table 2
Results of examination of different BGEs with regard to function *F*.

No.	Ammonium acetate [mmol/l]	Acetic acid [% *v/v*]	ACN [% *v/v*]	pH*	L_R	*t*	*CV*	*F*
1	50	0.1	25	5.31	5	21.12	3.00	0.39
2	50	0.1	5	5.08	3	30.52	1.44	0.21
3	50	0.01	25	6.34	5	18.92	1.39	0.95
4	50	0.01	5	5.88	3	17.87	1.56	0.32
5	5	0.1	25	4.69	2	16.53	0.29	0.83
6	5	0.1	5	4.23	2	23.84	1.18	0.14
7	5	0.01	25	5.41	3	10.53	0.70	1.21
8	5	0.01	5	5.47	2	13.58	2.72	0.11

In the next step, the sample solution was selected. Eight solutions used for injection of the sample: the chosen BGE, methanol, and mixtures of BGE and water, and ACN and water in ratios of 1:99, 1:9, and 1:3 *v/v* were tested. Samples dissolved in two reagents: methanol and BGE, gave efficient separation in terms of the shape of the peaks and their intensities. Then, the influence of the temperature in the sample garage on the sample solution (for samples dissolved in 20 μl of methanol or BGE) was investigated. Electrophoretic separations of dye mixtures kept at 10, 18 and 25 °C were performed after 0, 2, 4, 6, 10, 14 and 18 h for each temperature. In the case of dye mixtures dissolved in BGE, it was possible to carry out measurements after 4 and 18 h, for samples held at 18 and 10 °C, respectively. For samples held at 25 °C, the authors were only able to perform the first measurement at time "0". The same was observed for dye samples dissolved in methanol, for each temperature of the sample garage. This was the result of evaporation of the sample and lack of injection on the capillary. In routine analyses lasting up to several hours, earlier evaporation of the sample may be the cause of failure of experiments. Eventually, the authors selected the BGE as the sample solution, and the samples were held at 10 °C.

3.2. Extraction conditions and sample amount

In order to select the best extractant of black ink components from paper, three commonly used reagents: a mixture of pyridine and water 1:1 *v/v*, methanol, DMSO and the solution of BGE were tested with the use of sample I (see Table 1). As an example, the

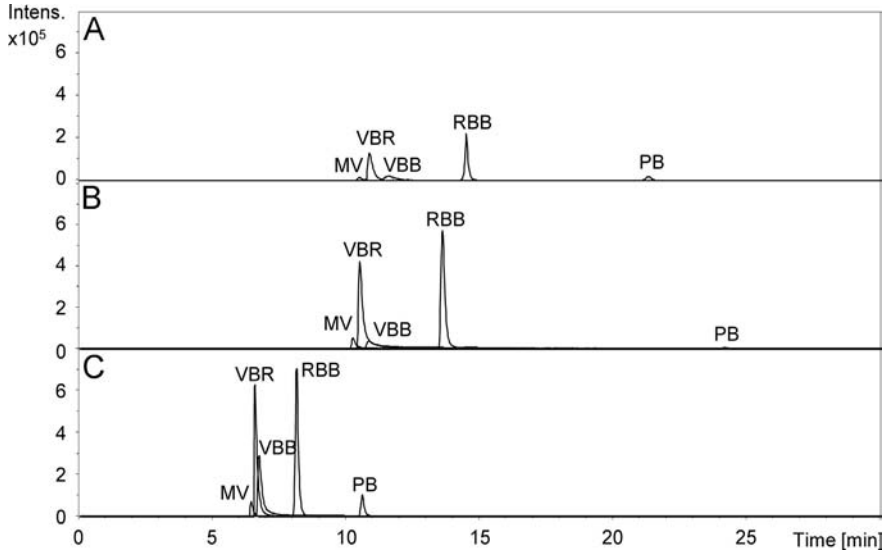

Fig. 1. Extracted ion electropherograms from CE-ESI-TOF-MS analyses of mixtures of five dyes: MV, PB, RBB, VBB, and VBR, in BGEs: (A) 1, (B) 6, and (C) 7, for CE conditions: capillary voltage +30 kV, measurement temperature 25 °C, sample storage temperature 10 °C, hydrodynamic injection: 0.7 psi, 7 s, and capillary i.d. 50 μm and 100 cm total length.

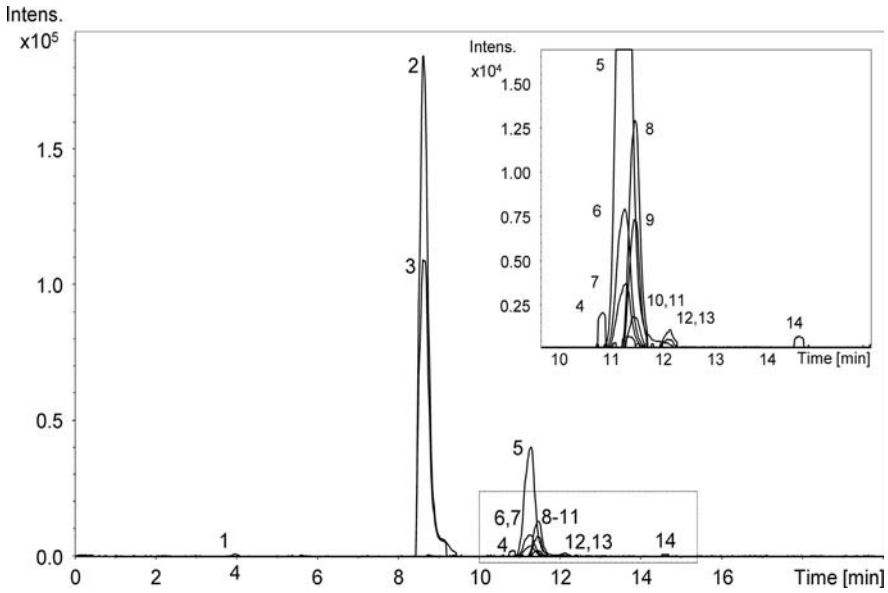

Fig. 2. Extracted ion electropherograms from CE-ESI-TOF-MS analysis of extract of sample I from paper with the use of DMSO in BGE 7, for CE conditions: capillary voltage +30 kV, measurement temperature 25 °C, sample storage temperature 10 °C, hydrodynamic injection: 0.7 psi, 7 s, and capillary i.d. 50 μm and 100 cm total length; *m/z* values of analytes: (1) 317.120, (2) 548.347, (3) 474.298, (4) 408.227, (5) 559.158, (6) 581.139, (7) 604.217, (8) 340.200, (9) 312.168, (10) 295.144, (11) 531.130, (12) 431.192, (13) 250.169, and (14) 350.186.

electrophoretic profile of sample I extracted from paper with the use of DMSO is shown in Fig. 2, with the section inside the dotted box enlarged in the upper right hand corner. Migration time at 8.62 min is characteristic for neutral molecules (extracted ion eletropherograms of $[M+H]^+$ ions for two neutral molecules are marked in Fig. 2 as peaks 2 and 3). Each tested reagent extracted analytes, labelled 1–13 in Fig. 2 from the printout. DMSO and the BGE extracted one additional compound: analyte 14 (with *m/z* value equal 350.186), in comparison with two other extractants. A larger number of extracted components of inks increased the discrimination capability of the method under development. The quantity of the extracted components, expressed as peak area, was also taken into account and in this respect similar results were obtained for both regents. The use of DMSO as the extraction reagent instead of BGE prolonged the examination of time of DMSO evaporation (to about 15 min). On the other hand, DMSO was chosen as the best extraction reagent for the MECC-DAD analysis of black inkjet ink extracts [10], and in the case of the sequence analysis of ink samples by CE-ESI-TOF-MS and MECC-DAD methods, DMSO was also selected as the extractant.

The amount of a sample (the number of chads with 0.63 mm diameter cut out from printout) required to perform effective extraction was chosen by monitoring the change of peak areas of 4 selected less intense peaks measured for sample I (*m/z* values of $[M+H]^+$): *m/z*=340.200, 604.217, 531.130, and 408.227. The peak areas of the ink components changed proportionally to the number of chads. For all compounds, the greatest increase in peak areas occurred up to 15 chads cut out from paper. However, extraction carried out for only 10 chads led to intense and symmetrical peaks for each of the analytes. Bearing in mind that the developed method is intended for forensic purposes, requiring as little destruction of the examined object as possible, 10 chads were finally established as sufficient for the extraction process. The number of 10 chads was confirmed by performing additional studies for samples VI, VIII, XII, and XIII, which demonstrated that there is no loss of any peak in comparison with samples obtained from 25 chads. The amount of added DMSO was also examined: for 5 and 10 μl added it was possible to separate 72.7% (*CV*=1.9%, for 5 samples) and 83.7% (*CV*=2.5%, for 5 samples) supernatant from paper, respectively. This confirmed the need for a larger volume of extractant, thereby prolonging the evaporation time slightly.

Two additional parameters having an impact on the analysed sample amount were verified – the pressure and time of hydrodynamic injection. Five variants of injection were tested: 0.4 psi for 4 and 10 s, 1.0 psi for 4 and 10 s, and 0.7 psi for 7 s. Strong band broadening was observed for the higher injection pressure, while lower repeatability of peak areas was obtained for shorter duration injections. The injection at a pressure of 0.7 psi for 7 s led to less band broadening and greatest repeatability of peak areas of the four monitored ink components (*CV* for 3 measurements in the range 0.8–18.3%, depending on the compound).

The separation conditions, the apparatus parameters and the extraction conditions selected during the method development process are presented in Table 3.

Extracted ion electropherograms for 5 analysed papers were recorded under selected conditions of the CE-ESI-TOF-MS method. Fig. 3 shows electrophoretic profiles collected for the studied papers and MS spectra acquired at migration times 4.74 and 9.69 min. Three out of five examined papers: Pollux, Rey, and Polspeed, all from one manufacturer and with paper density 80 g/cm^2, revealed the same electrophoretic profiles, presented in Fig. 3B–D, in contrast to Poljet (made by the same producer) and Presentation (by another producer), with profiles presented in Fig. 3E and A, respectively. All examined papers showed the presence of a characteristic MS spectrum at about 4.7 min. At the migration time of neutral molecules (9.69 min), a few paper components with *m/z* ratios (±0.003): 391.287, 415.214, 432.249, 460.271, and 522.203 were observed. The analytes present at this time, in particular, may interfere with neutral compounds extracted from printouts. The analytes labelled 3, with *m/z* values equal to 486.157 and 459.147 in Fig. 3A and E, and 539.105 present in Fig. 3B–D, originated most likely from optical brighteners added to the paper. The paper components may hinder analysis of inks; however, the MS detector allowed us to take into account only inkjet ink components in extracted ion electropherograms. The Polspeed paper, which had

the same characteristic electrophoretic profile as two other paper types and for which the smallest signal from optical brightener was obtained, was chosen for further study.

3.3. Precision

The random error of the *m/z* values was established. For this purpose, sample I was analysed 5 times for 5 days in the same CE-ESI-TOF-MS conditions, and the standard deviation of *m/z* values for every 5 measurements for analyte 2 (see Fig. 2) was calculated. The greatest value from 5 days of measurements, equal to 0.003, was designated to be the *m/z* similarity/difference threshold.

To determine the random error of migration times, 6 selected components of the sample extract with *m/z* ratios ($\pm$0.003): 317.120, 548.347, 312.168, and 559.158 (ink components) as well as 350.895 and 539.105 (paper components) were taken into consideration. Sample I was analysed. The repeatability results in relation to sampling (5 measurements for 5 samples from the same extract), extraction (5 measurements for 5 independently prepared extracts) and injection (5 measurements for 1 sample) was examined. The authors evaluated also the reproducibility of results in relation to sampling (25 measurements conducted over 5 days for 5 samples each day from the same extract), analyst (10 measurements conducted over 2 days by 2 analysts for 5 extracts prepared independently) and capillary (10 measurements conducted over 2 days on 2 different series of capillaries for 5 samples from the same extract). The results obtained are presented in Table 4.

The precision evaluated on the basis of absolute values of migration times was satisfactory. The results confirmed the slight changes in migration times of the individual compounds. There was a growth trend in *CV* values with increasing migration times of the compounds, as well as with decreasing signal intensities (e.g. compound with *m/z*=317.120). Carrying out several measurements for a single extract significantly improved the precision of the method (*CV* < 1%). The reproducibility of results for sampling and different analysts (under the conditions mentioned above) was also good (*CV* < 5% in most cases). However, the impact of the use of capillaries from different series and at different stages of working (a new capillary and a capillary after 30 days of taking measurements) on the migration times of the peaks was much stronger (the *CV* values exceeded 11% for the slowest migrating peak). This factor should be taken into account when comparing the results of measurements performed on various capillaries.

In order to improve the general precision of the method, measurements were carried out with an additional injection of an internal standard. The PB dye, whose analysis showed the presence of a symmetrical peak at 11.1 min, was selected as the internal standard out of five tested dyes (MV, PB, RBB, VBB, and VBR). 0.005 mg/ml solution of the PB dye in the BGE was injected into the capillary prior to injection of the analysed sample. The relative migration times of the components present in sample extracts were evaluated according to the following formula: $t_r = t_m/t_{st}$ (t_{st} is the migration time of the internal standard). The repeatability of results in relation to sampling, extraction and injection was in most cases

Table 3
Parameters and conditions of the developed CE-ESI-TOF-MS method.

CE	
BGE	1:3 *v/v* mixture of ACN:water, containing 5 mmol/l ammonium acetate and 0.01% *v/v* acetic acid
Capillary	A polyimide-coated fused silica capillary, 50 μm i.d., 375 μm o.d., 100 cm total length
Separation	Voltage +30 kV, temperature 25 °C
Sample temperature	10 °C
Injection	Hydrodynamic, 0.7 psi, 7 s
MS	
Ion source	Electrospray, positive ionisation
Sheath liquid	1:1 *v/v* isopropyl alcohol:water containing 0.2% *v/v* formic acid, 3 μl/min
Nebulizer	0.4 bar
Dry gas	4 l/min, 180 °C
Capillary voltage	4500 V
Inkjet ink sample	
Amount of sample	10 paper chads, each 0.63 mm diameter
Extractant	DMSO, 10 μl
Extraction process	15 min in ultrasonic bath, evaporation of DMSO in a stream of N_2 at 60 °C, centrifuge at speed 14,800 rpm for 5 min
Sample solution	BGE

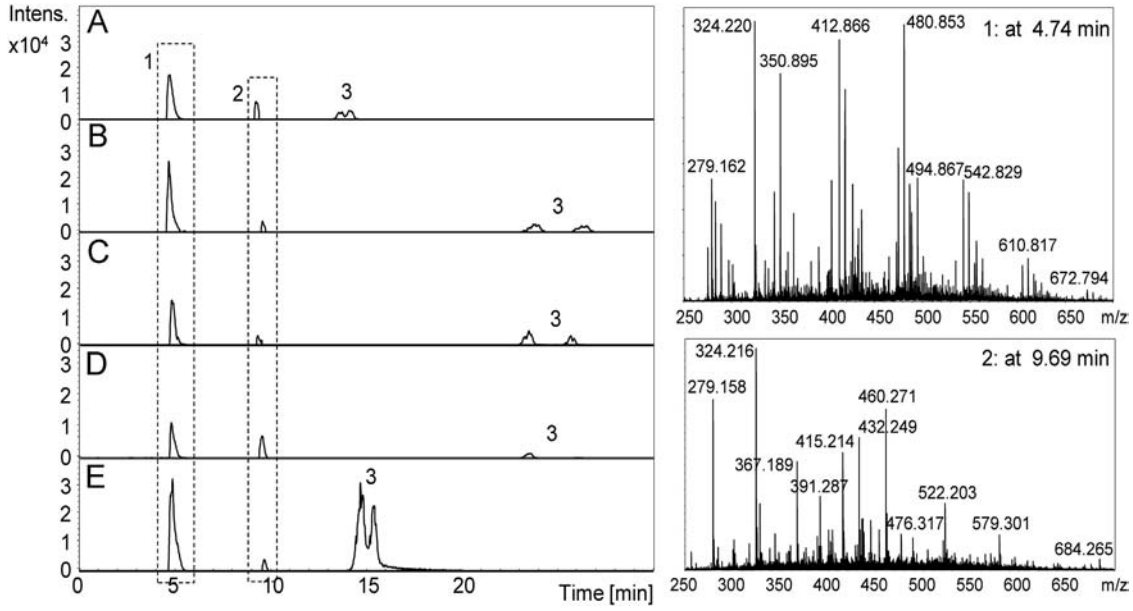

Fig. 3. Extracted ion electropherograms from CE-ESI-TOF-MS analysis of extracts of papers: (A) Navigator Presentation, (B) International Paper Pollux, (C) International Paper Rey, (D) International Paper Polspeed, and (E) International Paper Poljet and MS spectra at migration times: (1) 4.74 and (2) 9.69 min, recorded under selected apparatus parameters and separation conditions (see Table 3).

Table 4
The precision (*CV*) of the method, evaluated as repeatability (with respect to sampling (a), extraction (b), injection (c)), and reproducibility (with respect to sampling (d), analyst (e), and capillary (f)) of migration time (t_m) and relative migration time (t_r) (for details see text).

IS	t_m [min] or t_r	*m/z*	*CV* [%]					
			a	b	c	d	e	f
No	3.9	317.120	0.30	0.44	0.24	2.03	0.84	3.34
	4.6	350.895	0.48	0.58	0.82	3.32	2.18	1.56
	8.5	548.347	0.85	0.48	0.51	3.31	0.44	3.98
	11.1	559.158	1.49	0.84	0.31	4.52	0.72	4.16
	11.2	312.168	1.10	1.08	0.41	4.04	1.05	6.34
	18.0	539.105	2.02	2.23	0.49	6.29	5.85	11.45
PB dye	0.36	317.120	0.86	0.60	0.50	3.04	–	2.92
	0.44	350.895	0.66	0.82	1.23	2.98	–	4.93
	0.78	548.347	0.16	0.49	0.04	1.57	–	2.27
	1.0	559.158	0.25	0.29	0.22	1.49	–	1.96
	1.0	312.168	0.12	0.28	0.07	0.29	–	0.34
	1.7	539.105	0.58	1.02	0.11	3.08	–	5.83
Paper component	0.83	317.120	0.70	0.16	0.72	2.99	1.76	0.74
	1.0	350.895[a]	–	–	–	–	–	–
	1.8	548.347	0.62	0.28	1.23	2.55	2.42	1.02
	2.3	559.158	0.57	0.42	1.00	2.37	1.58	1.02
	2.3	312.168	0.69	0.62	1.18	3.13	1.24	1.63
	3.9	539.105	1.19	1.74	1.17	4.80	3.64	3.31

[a] Internal standard.

Table 5
The results of CE-ESI-TOF-MS analyses of examined ink samples.

Ink sample (figure no.)	*m/z* value (relative migration time, analyte no.)
I (2)	317.120 (0.85, 1), 548.347 (1.86, 2[a]), 474.298 (1.86, 3[a]), 408.227 (2.34, 4), 559.158 (2.43, 5), 581.139 (2.43, 6), 604.217 (2.43, 7), 340.200 (2.47, 8), 312.168 (2.46, 9), 295.144 (2.46, 10), 531.130 (2.44, 11), 431.192 (2.61, 12), 250.169 (2.61, 13), 350.186 (3.14, 14)
II	548.346 (1.83, 1[a]), 486.154 (2.66, 2), 360.125 (2.70, 3), 450.637 (2.77, 4)
III	548.344 (1.88, 1[a]), 559.155 (2.44, 2), 604.213 (2.44, 3), 581.135 (2.44, 4), 531.128 (2.46, 5), 508.953 (3.70, 6), 506.956 (3.71, 7)
IV	548.344 (1.89, 1[a]), 312.165 (3.78, 2)
V	320.288 (1.47, 1), 548.347 (1.81, 2[a]), 559.158 (2.37, 3), 604.213 (2.37, 4), 581.138 (2.37, 5), 531.127 (2.39, 6), 508.956 (3.55, 7), 506.957 (3.55, 8)
VI	548.350 (2.01, 1), 562.367 (2.71, 2)
VII	592.381 (2.56, 1), 562.368 (2.60, 2)
VIII (5A)	552.411 (1.93, 1[a]), 656.142 (4.46, 2), 477.107 (4.47, 3)
IX (5B)	556.440 (1.94, 1[a])
X (5C)	552.409 (1.89, 1[a]), 568.323 (2.49, 2), 524.294 (2.54, 3), 441.198 (2.65, 4)
XI	552.409 (1.84, 1[a]), 568.326 (2.40, 2), 524.294 (2.45, 3), 441.195 (2.55, 4)
XII	476.308 (1.91, 1[a])
XIII	552.412 (1.92, 1[a])

[a] Neutral component.

below 1%. Sampling reproducibility ranged from 0.29% to 3.08%. The greatest change was observed in the precision of the results from measurements carried out on different capillaries, for which, in the case of the slowest migrating component, *CV* was less than 6% (with a standard deviation of 0.1). Similar results (see Table 4), with a stronger reduction of *CV* values for analysis between different capillaries, were obtained using one of the paper components (with *m/z*=350.895) as the internal standard. In this case, there was no need for injection of an additional substance onto the capillary. Eventually, a decision on the use of the relative migration times – relative to one of the paper components – was taken.

3.4. Analysis of real samples

The developed and validated CE-ESI-TOF-MS method was applied to the separation of components of inks extracted from 13 black printouts (printed by printers equipped with original ink cartridges). The results of the performed CE-ESI-TOF-MS analyses for the examined samples are shown in Table 5. Each extracted component of the investigated samples was characterized by an *m/z* value and a relative migration time (relative to the paper component with *m/z* value 350.895). The results obtained for ink samples were compared with each other. The differentiation of inks was based on the number of significant peaks with individual *m/z* values, relative migration times and MS spectra (at the migration time of neutral molecules). Two analytes were considered as the same if the differences between their *m/z* values and relative migration times were less than or equal to 0.003 and 0.1, respectively.

For all inks, the most intensive peaks were revealed at the migration time of neutral compounds. As an example, MS spectra at the migration time of neutral molecules obtained for samples I, XII, and XIII are presented in Fig. 4. Mass distribution characteristic for polymer or surfactant components [4,12,14] was observed at this time. Based primarily on the MS spectra at the migration time of neutral molecules, inkjet inks of different manufacturers can be

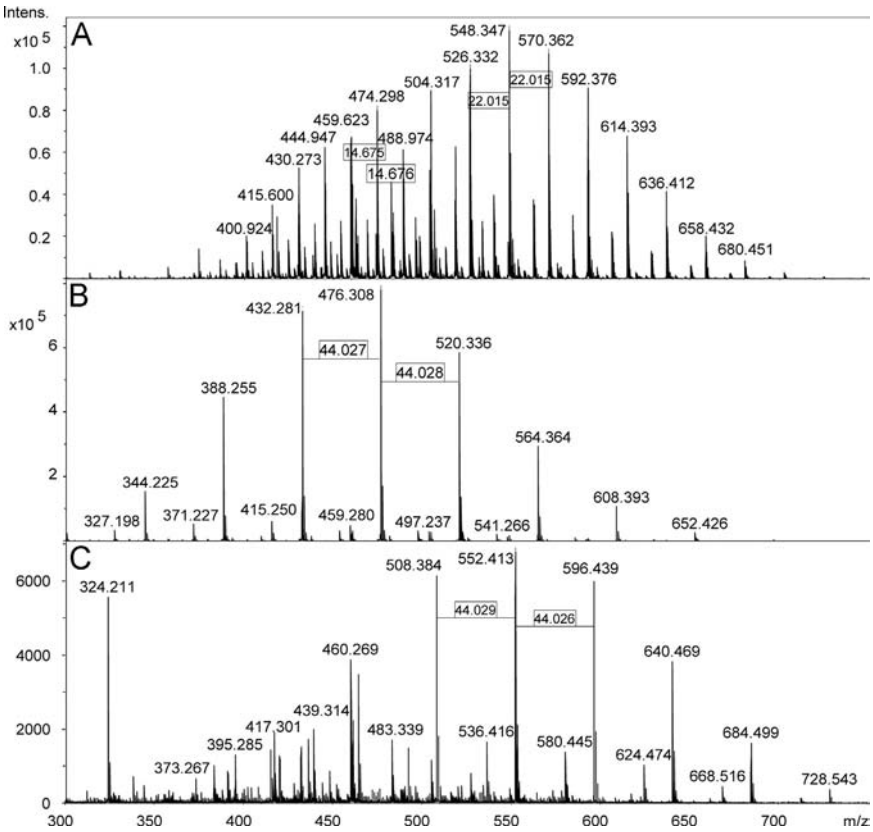

Fig. 4. MS spectra at the migration time of neutral molecules from CE-ESI-TOF-MS analyses of extracts of ink samples: (A) I (Hewlett-Packard), (B) XII (Lexmark), and (C) XIII (Epson) from paper for selected apparatus parameters and separation conditions (see Table 3).

distinguished from each other and group identification of respondent inks is possible. The MS spectrum shown in Fig. 4A was characteristic for all Hewlett-Packard inks. Particular *m/z* values differed by a constant value of 14.676 ± 0.003 for the first mass distribution and 22.015 ± 0.003 for the second mass distribution. In Lexmark inks, the MS profile presented in Fig. 4B was found. The last of the presented spectrum (Fig. 4C) appeared for both the Epson and Canon inks. In the case of the characteristic MS spectra of Lexmark, Epson and Canon inks, *m/z* values differed by 44.028 ± 0.003, most likely corresponding to C_2H_4O, which indicates the presence of polyethylene glycol with different terminal groups in the inks compositions. The examined Brother ink samples did not show such a characteristic MS profile.

It is worth noting that for the Hewlett-Packard inkjet inks, more ink components were extracted from paper in comparison with other ink producers. Epson and Lexmark inkjet inks (samples XII and XIII), in contrast to Hewlett-Packard inkjet inks, did not reveal any significant peak apart from peaks from the above mentioned polymer components.

In the case of inkjet inks from the same manufacturer, the greatest diversity of electrophoretic profiles was revealed for documents printed by Hewlett-Packard inkjet technology. Based on the *m/z* values and the relative migration times summarized in Table 5, Hewlett-Packard inks (samples I–V) can be easily differentiated. However, some analytes or groups of analytes are repeated for several inks, e.g. ions (± 0.003) 604.217, 581.139, 531.130, and 559.158 (derived most likely from red dye Sulphorhodamine B) present in three examined Hewlett-Packard inks (samples I, III, and V in Table 5). The presence/absence of other components of the inks allowed differentiation of samples. Examples are samples III and V, printed on different printer models but using inks with the same cartridge numbers, for which the same set of seven analytes and one additional compound present in ink sample V were observed.

The differences between original Canon inkjet inks were also meaningful. Extracted ion electropherograms from CE-ESI-TOF-MS analyses of three Canon inks (samples VIII–X) are presented in Fig. 5. Relative migration times and *m/z* values of each labelled component of samples VIII–X were collected in Table 5. Based on the results, Canon samples VIII and IX can be easily differentiated from each other and from samples X and XI. However, samples X and XI (printed on printers equipped with inks with the same cartridge numbers), revealed the same chemical composition. It is worth noting, that Canon ink IX exhibited a different MS spectrum for polymer components, as can be seen in Fig. 5D, from the other Canon inks, with the MS profile shown in Fig. 4C.

4. Conclusions

A modern combination of capillary electrophoresis and mass spectrometry was applied to the study of inkjet inks for forensic purposes for the first time. 10 chads from paper with 0.63 mm diameter were enough for successful examinations. The designated qualitative validation parameters of the developed method were satisfactory. However, an internal standard – Patent Blue dye – was necessary for using when the measurements were carried out on different days, in different capillaries (or at different stages of working). One of the paper components (extracted simultaneously with the components of inkjet inks from paper) can also be used as the internal standard. On the other hand, forensic document experts should be aware of the presence of additional components not derived from the examined samples in the tested extracts.

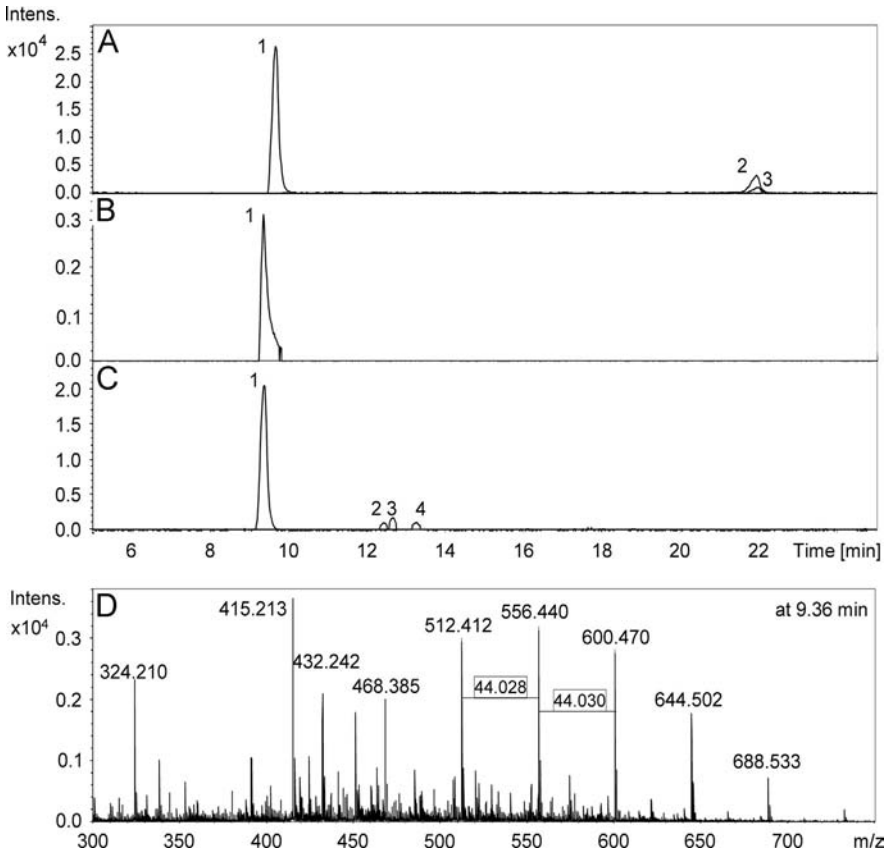

Fig. 5. Extracted ion electropherograms from CE-ESI-TOF-MS analyses of extracts of Canon inkjet ink samples (A) VIII, (B) IX, and (C) X (see Table 1) from paper, and (D) MS spectrum at migration time 9.36 obtained for sample IX, for selected apparatus parameters and separation conditions (see Table 3).

Qualitative analysis with the use of the developed method revealed differences in ink compositions. The electrophoretic profiles of the examined samples depended primarily on the inkjet ink producer and print technology. Since analysis of the main component of most black inkjet inks – pigment carbon black - was not possible, differentiation of ink samples was carried out solely on the basis of peaks originating from the additives present in the inks, e.g. polymer components. Printers using four inks for printing in black exhibited great diversity of electrophoretic profiles of ink extracts from paper. This was observed for Hewlett-Packard ink samples, for which red dye was revealed in extracts from black printouts. Further changes in the extracted ion electropherograms may be the result of the use of increasingly popular substituted inkjet inks although this needs to be confirmed by systematic studies.

The study revealed the presence of a characteristic mass spectrum (at the migration time of neutral species) originating from a surfactant or polymer, e.g. polyethylene glycol with different end groups, for most of the investigated inks. The mass distribution of the additive was distinctive for Hewlett-Packard and Lexmark, and in some cases for Epson and Canon inkjet inks, and allowed for group identification of ink samples. On the other hand, a lack of such an MS profile may indicate that the examined ink is produced by Brother.

The separation effectiveness of the CE technique combined with the identification capabilities and high sensitivity of the MS technique offer great opportunities in comparative analysis of inkjet inks for forensic purposes. The versatility of the method not only allows reliable analysis of black inkjet inks, but also creates possibilities of analysis of other types of inks, such as writing inks.

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