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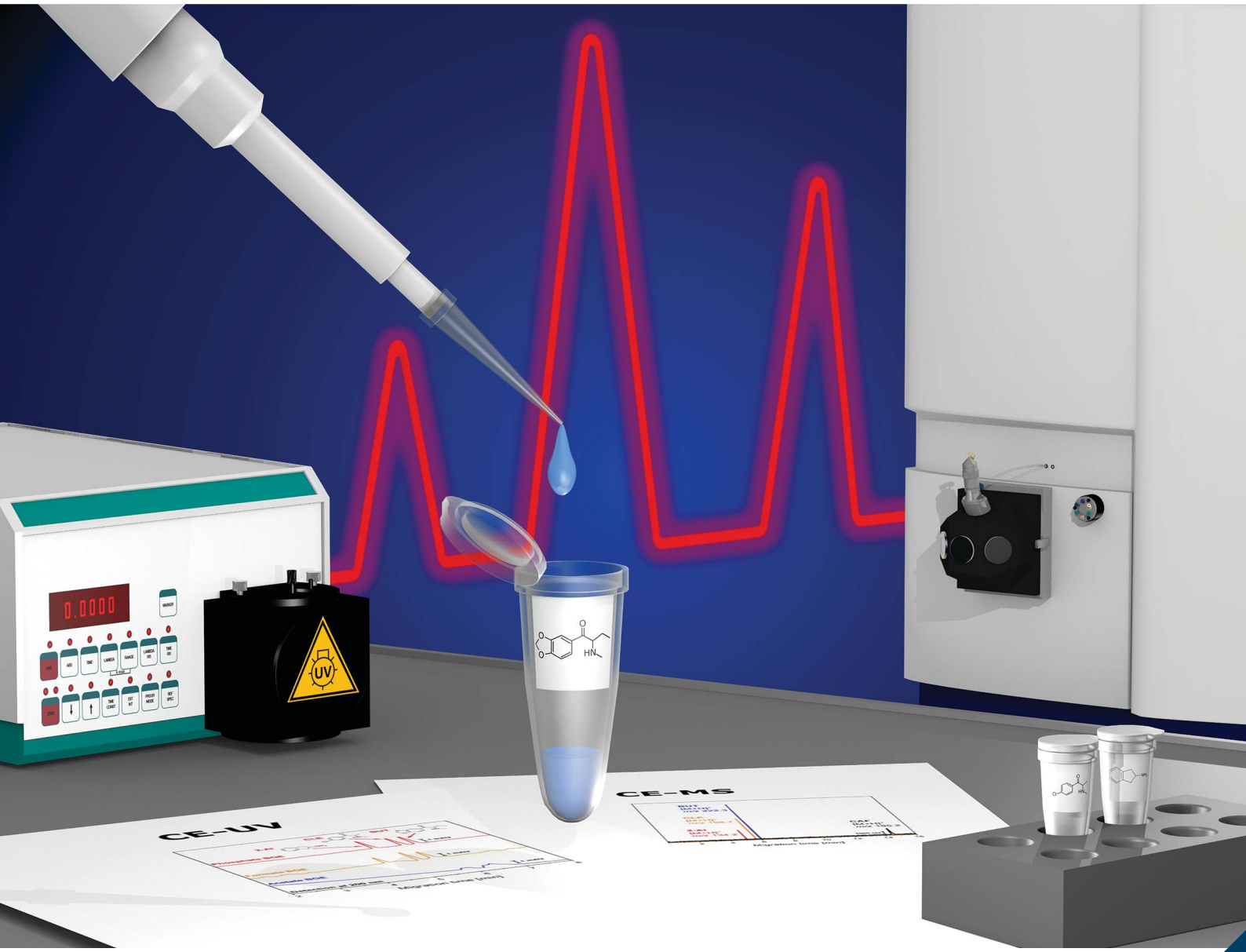

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PAPER

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Evaluating possibilities for the determination of novel psychoactive substances in human saliva using capillary electrophoresis with UV detection and mass spectrometry†

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Novel psychoactive substances are an emerging threat in the field of illegally consumed compounds. The subgroup of synthetic cathinones increased in popularity due to their great variety and the lack of powerful instrumental methods for their efficient monitoring. In the context of detecting drug-related crimes, saliva is a promising sample due to the presence of significant amounts of the respective species and easy and non-invasive accessibility compared to blood or urine samples. Herein, we report two powerful instrumental approaches based on the high separation efficiency and low sample consumption of capillary electrophoresis, combined with commonly used detection principles, namely mass spectrometry and UV detection. Mass spectrometry offers high selectivity and the possibility to identify comigrating analytes but comes with some instrumental challenges and high investment costs, while UV detection provides simple coupling to capillary electrophoresis and cost-efficiency. Moreover, we developed a simple and fast pretreatment protocol for the handling of directly collected human saliva samples for subsequent injection into capillary electrophoretic systems. In this study, we successfully detected butylone and clephedrone, part of the synthetic cathinone family, and 2-aminoindane, which are all listed as psychoactive substances. Furthermore, the methods were characterized in terms of detectability, reproducibility, and limits of detection (6–15 μM). Based on this, an application in the forensic context using CE-UV was simulated for 2-aminoindane and butylone at a concentration of 10 μM (20 μM).

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1 Introduction

In recent years, the rise of new psychoactive substances (NPSs) has changed drug abuse by introducing compounds that avoid traditional detection methods and threaten public health. Designed to mimic the psychoactive effects of commonly known drugs, NPSs often exploit legal loopholes, as their regulation does not keep up with their rapid emergence.[1] Moreover, their overall impact on human health is not sufficiently investigated, increasing the potential for harm. For example, synthetic cathinones (SCs)—the second-largest group of NPSs—first appeared on the drug market in 2003 and have since gained popularity as ecstasy alternatives. Alarmingly, between 2017 and 2020, the literature reported 31 different SCs being involved in 75 fatalities.[2] The rapid spread of NPSs around the world poses difficulties for law enforcement and forensic science, requiring the development of new analytical methods to keep up with the constantly changing drug market.

Not only the quick changes in NPSs structure but also the matrices used for NPSs analysis present a multitude of analytical challenges. Therefore, powerful instrumental approaches are of central importance. Spectroscopic methods are beneficial for tablets and different solid street samples due to their portability and rapid analysis capabilities,[3,4] while for mixed samples (*i.e.*, biological samples and samples containing multiple NPSs), the implementation of separation techniques can be advantageous. Gas and liquid chromatography coupled with mass spectrometry are commonly used for the identification of SCs in blood,[5,6] urine,[7,8] hair,[9] and oral fluid.[10,11] Among these, oral fluid is a particularly advantageous matrix due to its non-invasive collection and close correlation with recent drug abuse. In general, concentration in the range from 10^{-7} to 10^{-5} M can be found in oral fluid after administration of SCs. The exact concentration detected strongly depends on the dose and way the substance is administered. Reports for oral or intranasal consumption of different amounts of substance for members of the SC family led to saliva concentrations of

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6 to 80 μM for mephedrone[12] and 0.1 to 10 μM for methylone.[13] Following the oral intake of 100 mg of clephedrone, peak saliva concentrations were observed between 28.65 and 30.35 μM, occurring two to three hours post-consumption.[14] While chromatographic methods so far have dominated the analysis of NPSs, capillary electrophoresis (CE) could be a promising alternative due to its low investment costs, high separation efficiency, and low sample and reagent consumption.[15] Therefore, several CE methods have been reported in the context of drug monitoring. For example, Mazina *et al.*[16] used CE with LED-induced native fluorescence detection for cannabinoids in oral fluids. With this setup, it was possible to analyze the two main cannabinoids within 10 minutes, including sample collection, preparation, and CE analysis. Similarly, Saar-Reismaa *et al.*[17] used CE with deep UV excited fluorescence for *in situ* determination of illegal drugs in oral fluid. Despite these advancements, the use of CE for SC analysis remained limited primarily to enantiomeric separations and involved complex instrumental setups and complex background electrolytes (BGEs). Řezanka *et al.*[18] have used cyclodextrins as chiral selectors for enantioseparation of mephedrone and its metabolites. In another case, a supramolecular deep eutectic solvent was used as a chiral selector for the enantiomeric separation of nefopam and five SCs.[19] Moreover, many applications utilizing electrophoretic techniques (mainly, capillary and chip electrophoresis) in the context of saliva analyses, coupled with sophisticated sample treatment protocols like microdialysis, solid-phase, liquid-phase, and membrane-based extraction, have been reported.[20,21] Like its chromatographic counterparts, CE could also be coupled with MS (CE-MS), which offers good sensitivity and selectivity for a variety of substances. Furthermore, MS offers the possibility of providing information about co-migrating analytes.[22,23] However, CE-MS involves high instrumental effort and monetary investment, and its use is limited to BGE systems consisting exclusively of volatile constituents. UV detection also provides a powerful and widely used detection principle in modern CE due to its cost-efficiency, robustness, and user-friendliness, and can be used in combination with a great variety of BGE systems. Consequently, CE-UV offers a simple but efficient instrumental setup, which is particularly well-suited for the rapid and cost-effective detection of NPSs in various biological matrices, including oral fluids. To our knowledge, no CE method for the determination of various synthetic cathinones in oral fluid (including human saliva) has been described so far.

In this work, we evaluated the application of two powerful methods based on CE, namely CE-MS and CE-UV, for their suitability for the determination of SCs (represented in this study by butylone and clephedrone) in human saliva. The implementation of dimethylbiguanidine (DMBG) as an internal standard and a straightforward sample preparation technique of human saliva allowed reliable and fast analysis within 15 min, making the developed methods highly suitable for high-througput analysis. Here, CE-UV could be used for fast and cheap routine determinations, and CE-MS is the method of choice when peak purity needs to be ensured.

2 Experimental

2.1 Reagents and materials

The following chemicals were all of analytical grade: butylone hydrochloride (BUT, $pK_a = 8.1$) and clephedrone hydrochloride (CLE, $pK_a = 8.0$) were supplied by Alfarma s.r.o. (Cernošice, Czech Republic). Caffeine (CAF) was bought from ABCR Chemicals (Karlsruhe, Germany). 2-Aminoindane hydrochloride (2-AI, $pK_a = 9.8$) and dimethylbiguanidine (DMBG) were purchased from Sigma Aldrich (St. Louis, USA). Formic acid, hydrochloric acid (1 M), phosphoric acid (85%), sodium hydroxide, and sodium dihydrogen phosphate were purchased from Merck (Darmstadt, Germany). Ammonium formate was supplied by Alfa Aesar (Karlsruhe, Germany). Acetic acid and 2-propanol (LC-MS grade) were purchased from Carl Roth (Karlsruhe, Germany). Ultrapure water was provided by a Milli-Q Advantage A10 system (Merck Darmstadt, Germany). Fused silica capillaries (50 μm inner diameter, 365 μm outer diameter, polyimide coated, length 60 cm) were purchased from Polymicro Technologies (Phoenix, USA).

Human saliva was directly collected from the authors.

The pK_a values of 2-AI, BUT, and CLE were approximated using software from Chemaxon (Budapest, Hungary).

2.2 Prepared solutions

CE separation was carried out in the following BGEs: formate (100 mM formic acid and 100 mM ammonium formate in ultrapure water, measured pH 3.7), phosphate (100 mM phosphoric acid and 100 mM sodium dihydrogen phosphate in ultra-pure water, measured pH 2.1), and acetate (100 mM acetic acid and 100 mM ammonium acetate in ultra-pure water, measured pH 4.5). For the pH measurements, a WTW 538 pH meter from Xylem (Rye Brook, USA) was used. For the investigation of the detection wavelength and the analytical performance, samples containing 2-AI, BUT, CAF, and CLE were prepared freshly each day using pretreated human saliva and analyte from the respective stock solution (1 mM, in H_2O). Human saliva samples were collected with the consent of a volunteer. Saliva was pretreated in the following way: Human saliva was directly collected (9 mL). Afterward, the saliva was diluted with 1 M hydrochloric acid (3 : 1, v/v) and centrifuged at 10 000 rpm for 5 min for protein removal. The clear liquid was collected and used as pretreated saliva.

2.3 Instruments and methods

2.3.1 CE-UV. Analyses were conducted using a laboratory-constructed CE device (MINI CE)[24] in combination with a single wavelength detector, namely the ECD 2600 CE UV detector from ECOM (Prague, Czech Republic). The injection was controlled by a siphon effect driven by a height difference of 10 cm for 15 s into a fused silica capillary (I.D. 50 μm, length 60 cm, effective length 50 cm). CE separation was carried out at 15 kV. During the measurements the electrophoretic current was between 90 and 100 μA. Different wavelengths were used for the optimum detection of each analyte. The CE runs were stopped after 8 min to save analysis time, and the capillary was flushed for 1 min with separation BGE using negative pressure

at the outlet while high voltage remained applied. This approach was chosen to keep the CE conditions as stable as possible while flushing the capillary. All measurements were carried out at different detection wavelengths from 190 to 270 nm. At the beginning of each measurement day, the capillary was conditioned according to the following protocol: flushed for 10 min with 0.1 M sodium hydroxide solution, followed by 5 min water, and concluded by 30 min separation BGE.

2.3.2 CE-MS. For CE-MS, the MINI CE system was coupled to a micrOTOF from Bruker Daltonics (Bremen, Germany) equipped with a coaxial sheath-flow ESI interface from Agilent Technologies (Santa Clara, USA) *via* a 50 cm long capillary (I.D. 50 μm). The injection was controlled by a siphon effect driven by a height difference of 10 cm for 15 s. During the measurements, the electrophoretic current was between 90 and 100 μA. CE separation was carried out at 15 kV. The mass spectrometer was operated with the following parameters: nebulizer gas (N_2) pressure 0.3 bar, dry gas (N_2) flow 2.5 L min^{-1}, dry gas temperature 240 °C, and *m*/*z* 50–400. The sheath liquid had a flow rate of 8 μL min^{-1}. For these studies, the micrOTOF was operated in positive ion polarity mode, and a sheath liquid based on H_2O : 2-propanol : formic acid (49.95 : 49.95 : 0.1, v/v/v) was used. Data Analysis 4.0 SP1 from Bruker Daltonics (Bremen, Germany) and MZmine 3 (ref. 25) were used for data handling. At the beginning of each measurement day, the capillary was conditioned according to the following protocol: flush for 10 min with 0.1 M sodium hydroxide solution, followed by 5 min water, and concluded by 30 min separation BGE.

2.3.3 Photometry. A Cary 50 spectrophotometer from Varian (Palo Alto, USA) equipped with a fused silica cuvette (QS, high performance 200–2500 nm, 10 mm) from Hellma GmbH & Co. KG (Müllheim, Germany) was used to measure UV spectra of water, formate BGE, and phosphate BGE.

2.4 Determination of 2-aminoindane and butylone in forensics

DMBG was used as an internal standard for the determinations under forensic conditions. For both determinations, the standard solutions containing 2-AI (BUT) in the concentration range of 5 to 40 μM and 40 μM DMBG were prepared using stock solutions (1 mM in H_2O). To account for the acidic conditions in the saliva samples, these calibration solutions were prepared with H_2O : 1 M hydrochloric acid (3 : 1, v/v). The collected saliva was directly spiked with 10 μM (20 μM) 2-AI or BUT and 53.3 μM DMBG. Afterward, the same saliva treatment was carried out as mentioned above, resulting in 7.5 μM (15 μM) 2-AI (BUT) and 40 μM DMBG in the saliva samples after sample preparation. The measurements related to 2-AI were carried out using phosphate BGE at 190 nm, while BUT detection was performed at 240 nm in formate BGE.

3 Results and discussion

3.1 Migration behavior and detection of 2-aminoindane, butylone, and clephedrone

As a first step, the migration behavior of 2-AI, BUT, and CLE was investigated in different separation BGEs commonly used in CE. CE-UV was chosen for this step due to the simple instrumental setup and user-friendliness. Acetate, formate, and phosphate systems were tested. Each BGE consisted of 100 mM electrolyte (ammonium acetate, ammonium formate, or sodium dihydrogen phosphate) and 100 mM of the corresponding acid. The respective electropherograms of a mixture of 100 μM 2-AI, BUT, and CLE are shown in Fig. 1. Successful CE separation could be achieved in formate (measured pH 3.7) and phosphate (measured pH 2.1) BGE. The use of acetate BGE (measured pH 4.5) led to insufficient separation, suggesting a pH-dependence for CE separation of 2-AI (pK_a = 9.8), BUT (pK_a = 8.1), and CLE (pK_a = 8.0), whereby media with pH less than 4 are required. This is also reflected by the anlytes' pK_a values, characterizing them as weak acids in their protonated form, which is present at pH 3.7 and pH 2.1. For formate and phosphate BGE, each compound showed a distinct migration behavior in the migration window for positively charged analytes and could be identified by the respective signal. A further decrease in pH (tested by the change from formate (pH 3.7) to phosphate (pH 2.1) BGE, see Fig. 1) did not bring additional advantages for CE separation. Additionally, the similar migration times in both media indicate independence of the BGE system (formate *vs.* phosphate) and migration behavior, at low pH. Low pH values like 3.7 and 2.1 lead to low EOF activity, leaving the electrophoretic mobility of the individual species and gravity as the main driving forces for migration of the analytes, which is independent of the choice of separation medium (formate or phosphate) in this situation. However, the measurement utilizing phosphate BGE showed a significantly better signal-to-noise ratio at 200 nm. This could be attributed to the lack of intrinsic UV absorption of phosphate compared to formate at wavelengths below 230 nm (see Fig. S1, ESI†), leading to lower limits of detection (LODs, see Table 1) using phosphate BGE compared to formate BGE for all analytes. Therefore, the phosphate BGE should be combined with CE-UV for optimum detection wavelengths below 230 nm (for example, 2-AI at optimum conditions). At higher wavelengths, the use of formate had no negative influence on the sensitivity of the determination and could be used for the optimum determination of BUT and CLE as well. The respective electropherograms can be found in Fig. 3 (right panel). Furthermore, the formate BGE system bears the additional advantage of consisting only of volatile constituents and can be, therefore, directly applied in CE-MS without further modification, saving time and cost for method development. All following characterization steps were carried out using formate BGE due to its ability to serve as BGE in CE-MS and CE-UV, respectively. Fig. 2 illustrates CE-MS (left) and CE-UV (right, red electropherogram) measurements using formate BGE. A mixture of 100 μM 2-AI, BUT, and CLE was investigated using caffeine (CAF) as an EOF-marker. For CE-MS, the *m*/*z* values for the extracted ion electropherograms were derived from the mass spectra of the respective compounds (see Fig. S2A–C, ESI†). For all compounds, the predominant signal could be attributed to the protonated molecule $[M + H]^+$. For CE-UV, the detection wavelength was set to 270 nm to ensure optimum CAF detection. Both methods showed similar CE separation patterns and detectability of 2-AI, BUT, and CLE in

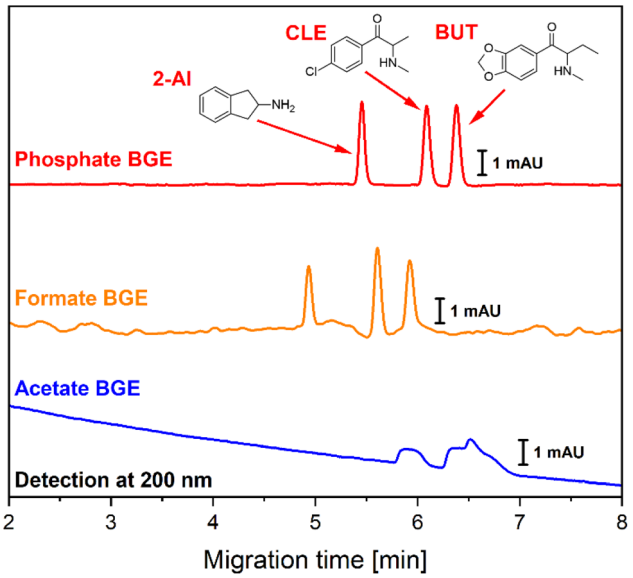

Fig. 1 Migration behavior of 2-AI, BUT, and CLE in different separation BGEs. Electropherograms of a 100 μM mixture in acetate BGE (100 mM ammonium acetate and 100 mM acetic acid, blue), formate BGE (100 mM ammonium formate and 100 mM formic acid, orange), and phosphate BGE (100 mM sodium dihydrogen phosphate, 100 mM phosphoric acid, red). UV detection at 200 nm. CE conditions: separation voltage 15 kV, capillary ID 50 μm, capillary length 60 cm (effective length 50 cm). The injection was controlled by a siphon effect driven by a height difference of 10 cm for 15 s.

the migration window for cationic analytes, suggesting protonation in formate BGE. Additionally, all analytes could be identified using their characteristic migration times and m/z values. Moreover, CE-UV was tested for samples dissolved in separation BGE and pretreated human saliva (Fig. 2, right panel, black electropherogram). Here, human saliva was mixed with 1 M HCl (3 : 1, v/v) and centrifuged for 5 min. The clear centrifugate was used as pretreated human saliva, as human saliva is a matrix containing a great variety of proteins and other residues that could influence CE separation and UV detection. The sample dissolved in pretreated human saliva showed no significant change in migration behavior or signal intensity of the analytes compared to the sample dissolved in separation BGE. Moreover, in the electropherogram of a sample in human saliva, more signals in the migration window for positively charged analytes could be observed between 10 and 15 min, indicating additional saliva constituents that had been successfully separated from the analytes. To account for the complex matrix, which is proposed by human saliva, all the following characterization steps for CE-UV were carried out using analytes dissolved in pretreated saliva samples. The separation for all subsequent measurements could be shortened from 20 min to 8 min by flushing after 8 min as all analytes of interest migrated between 5 to 7 min.

3.2 Investigation of the detection wavelength in CE-UV

Knowing the migration characteristics of 2-AI, BUT, and CLE, the UV absorption behavior for each compound was investigated as the next step. For this purpose, a sample containing 100 μM 2-AI, BUT, and CLE dissolved in human saliva was measured at different wavelengths in the range of 190–270 nm. To identify the optimum detection wavelength for each compound, the peak heights for the signal of each analyte were plotted against the corresponding wavelength, as shown in Fig. 3 (left panel). Beginning at 190 nm, all analytes showed UV absorption. The intensity rapidly decreased for 2-AI. At 240 nm, only BUT and CLE gave a UV response. The absorption of BUT decreased after 240 nm. At 260 nm, only CLE showed significant UV absorption. With this approach, the optimized detection wavelengths under CE conditions could be identified as 190 nm for 2-AI, 240 nm for BUT, and 260 nm for CLE. The electropherograms for a 100 μM mixture of the analyte at each optimum wavelength are shown in Fig. 2 (right panel). An increase in noise can be observed for lower wavelengths, especially for 190 nm, as already discussed in Section 3.1. This could be attributed to the intrinsic UV absorption of the BGE and lead to a loss of sensitivity at the lower end of the wavelength interval, which was investigated. With this knowledge, each compound could be determined at the optimum wavelength to provide the most sensitive determination possible. 200 nm could be seen as a compromise of being able to detect all analytes at the same time and keeping the noise level at the lowest stage possible. This wavelength could be used for screening analysis, followed by the determination of the respective analytes at the optimum wavelengths.

3.3 Analytical performance of CE-UV and CE-MS

With optimized detection wavelengths, the following aspect addressed in this study was the detectability of 2-AI, BUT, and CLE, applying CE-UV and CE-MS. For CE-UV, the limits of detection (LODs) of all analytes at optimal wavelengths for

Table 1 LODs for S/N = 3 of 2-AI, BUT, and CLE at 190, 200, 240, and 260 nm and their standard deviation (relative standard deviation in brackets). Concentrations in a range of 10 to 50 μM were investigated (n = 3)

		LODs [μM]					
Detection method		2-AI		BUT		CLE	
UV/VIS	190 nm	14.7 ± 0.2	(1.4%)	—	—	29.8 ± 0.5	(1.7%)
	200 nm	32.0 ± 0.6	(1.9%)	20.0 ± 0.3	(1.5%)	21.6 ± 0.4	(1.9%)
	240 nm	—	—	4.2 ± 0.2	(4.8%)	8.1 ± 0.3	(3.7%)
	260 nm	—	—	—	—	4.0 ± 0.2	(5.0%)
ESI-MS		16 ± 1	(6.3%)	5.7 ± 0.3	(5.3%)	6.2 ± 0.5	(8.1%)

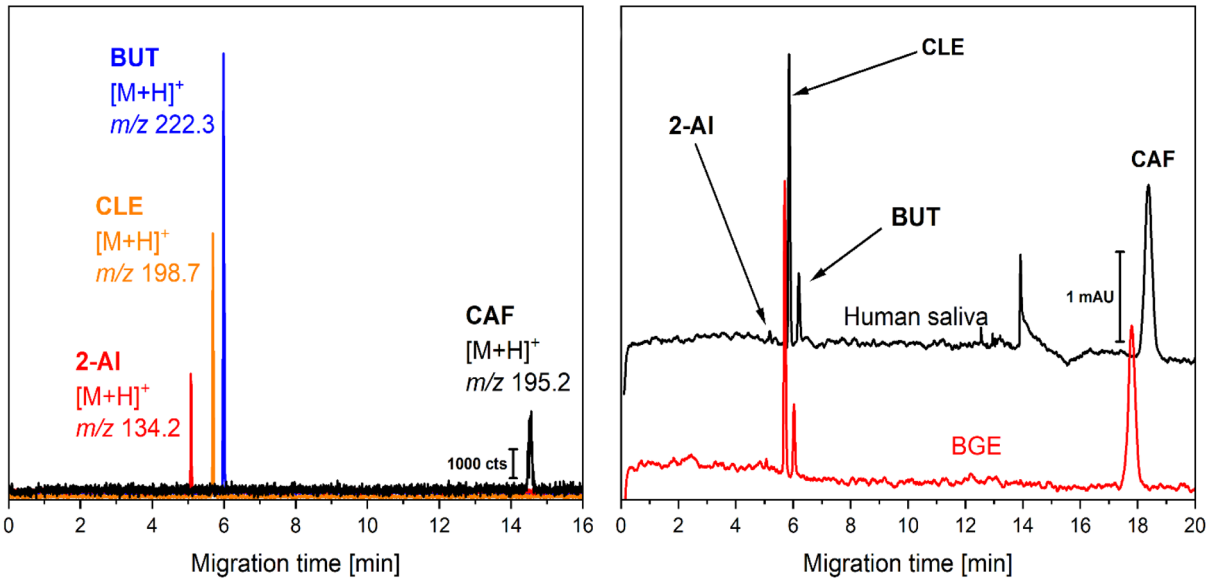

Fig. 2 Measurements of a mixture of 100 μM 2-AI, BUT, and CLE, 500 μM CAF added as an EOF marker. The CE separation was carried out in formate BGE (100 mM ammonium formate and 100 mM formic acid). CE-MS (left): Extracted ion electropherograms for 2-AI (*m/z* 134.2, red), BUT (*m/z* 222.3, blue), CAF (*m/z* 195.2, black), and CLE (*m/z* 198.7, orange). The corresponding *m/z* values were taken from the respective mass spectra in Fig. S1 in the ESI.† CE-UV (right): Electropherograms measured at 270 nm (optimum for CAF detection). Analytes dissolved in BGE (red) and analytes dissolved in human saliva after protein removal (black). CE conditions: separation voltage 15 kV, capillary ID 50 μm, capillary length 60 cm (effective length 50 cm). The injection was controlled by a siphon effect driven by a height difference of 10 cm for 15 s.

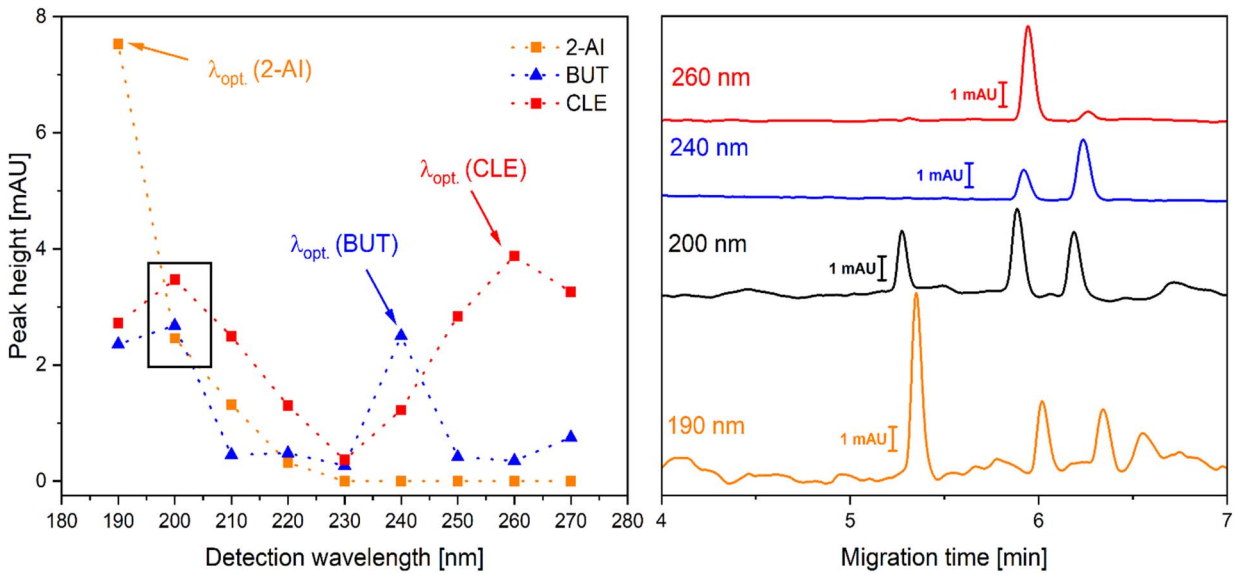

Fig. 3 CE-UV measurements of the same sample at different wavelengths (190–270 nm). The sample mixture contained 100 μM 2-AI, BUT, and CLE in pretreated human saliva. The separation BGE contained 100 mM ammonium formate and 100 mM formic acid. Left: Dependence of peak height and detection wavelength for 2-AI (orange), BUT (blue), and CLE (red). Optimum detection wavelengths ($\lambda_{opt.}$) for each compound and the optimum wavelength for the simultaneous detection of all analytes (200 nm, black box). Right: Electropherograms of the sample mixture for the optimum detection wavelengths for each compound. 190 nm (orange), 200 nm (simultaneous detection of all analytes, black), 240 nm (blue), 260 nm (red). CE conditions: separation voltage 15 kV, capillary ID 50 μm, capillary length 60 cm (effective length 50 cm). The injection was controlled by a siphon effect driven by a height difference of 10 cm for 15 s.

detecting each single compound were investigated, as well as for 200 nm using solutions of 2-AI, BUT, and CLE in pretreated human saliva. For CE-MS, measurements were performed using a coaxial sheath-flow interface without any further optimization. For the determination of the LODs for both methods, solutions in the range of 10–50 μM were used. All LODs were determined for an S/N of 3 and are listed in Table 1.

Table 2 Migration times, peak heights, and peak areas (mean and standard deviation, relative standard deviation in brackets) for measurements of a mixture of 100 μM 2-AI, BUT, and CLE. UV detection at 200 nm ($n = 10$)

Detection method		Migration time [min]		Peak height [mAU]		Peak area [mAU min]	
UV detection (200 nm)	2-AI	5.30 ± 0.03	(0.6%)	2.5 ± 0.2	(8.0%)	0.19 ± 0.02	(10.5%)
	BUT	6.34 ± 0.05	(0.8%)	2.7 ± 0.1	(3.7%)	0.29 ± 0.03	(10.3%)
	CLE	6.00 ± 0.04	(0.7%)	3.5 ± 0.1	(2.9%)	0.25 ± 0.02	(8.0%)
Detection method		Migration time [min]		Peak height 10^3 Cts		Peak area 10^3 Cts min	
ESI-MS	2-AI	5.26 ± 0.06	(1.1%)	9 ± 1	(11.1%)	0.4 ± 0.1	(25.0%)
	BUT	6.33 ± 0.07	(1.1%)	24 ± 4	(16.7%)	1.4 ± 0.2	(14.3%)
	CLE	5.98 ± 0.07	(1.2%)	15 ± 1	(6.7%)	0.8 ± 0.1	(12.5%)

For CE-MS and CE-UV, comparable LODs could be achieved in the lower μM range.

For CE-UV, the lowest LOD for each substance, ranging from 4.0 to 15 μM, was found at the optimum detection wavelength indicated in Fig. 3. For 190 nm, it was not possible to determine a LOD for BUT using S/N = 3 in the investigated concentration range due to high noise levels. Additionally, a higher LOD of 2-AI at its optimum conditions could also be related to increased noise. Intrinsic absorption behavior of formate at 190 and 200 nm could lead to an increase in noise and, therefore, to a loss of sensitivity. An additional CE-UV investigation for 2-AI at 190 nm in phosphate separation BGE resulted in an LOD of 5.4 μM ± 7.4%, which is comparable to BUT and CLE at their optimum conditions. Furthermore, in the case of 200 nm, similar LODs between 20 and 30 μM for 2-AI, BUT, and CLE could be found. It was not possible to determine LODs for 2-AI at 240 and 260 nm and for BUT at 260 nm due to a lack of UV absorption of the analytes at these wavelengths. Another important aspect is the reproducibility of the CE-UV and CE-MS measurements. For this purpose, ten consecutive measurements of a 100 μM solution of 2-AI, BUT, and CLE were carried out. Migration times, peak heights, peak areas, and their respective relative standard deviation are summarized in Table 2. The detection wavelength was set to 200 nm to provide information about all analytes simultaneously. For CE-MS, similar LODs could be found for all analytes compared to CE-UV. In this case, 2-AI also showed with 15 μM a significantly higher LOD compared to BUT and CLE (6 μM). A reason for this behavior could be a lower ionization efficiency for 2-AI. These LODs are in the concentration intervals for similar substances in clinical samples reported in the literature.[12–14] Therefore, this method could be used to determine 2-AI, BUT, and CLE at the same concentration levels in saliva after their consumption. Regarding similar values for the LODs, CE-MS showed no advantage in terms of sensitivity compared to CE-UV. Consequently, both methods are suitable for sensitive SC determinations in human saliva. However, peak purity monitoring is only possible using CE-MS. For the investigation of the reproducibility of CE-UV and CE-MS determinations, ten consecutive measurements of a 100 μM mixture 2-AI, BUT and CLE were carried out (see Table 2), Migration time, peak hight and peak area showed for both methods only a small relative standard deviation in the lower percent range which makes both methods suitable for reliable quantitations. Regarding these parameters, the analytes could be identified by their characteristic migration time and quantified by the respective peak height and peak area.

3.4 Determination of 2-aminoindane, butylone, and clephedrone in drug monitoring and forensics

Due to their psychoactive properties, 2-AI, BUT, and CLE fall into the category of legally regulated and prohibited substances. For these substances, saliva is the most straightforward available evidence to determine their abuse. Therefore, strong detection methods are needed in drug monitoring and forensics. CE-UV detection has great potential in this field.

In this section, we present an exemplary study of SC determination in human saliva. To ensure the best performance possible, an internal standard was added. Dimethylbiguanidine (DMBG) was evaluated as a suitable candidate. Therefore, the migration behavior of DMBG was investigated for a sample mixture of 100 μM 2-AI, BUT, CLE, and DMBG using phosphate BGE. The resulting electropherogram is shown in Fig. 4. Here, a distinct migration behavior and UV absorption at 200 nm could be observed for all species. The DMBG signal appeared as the first peak in the electropherogram, followed by 2-AI, CLE, and, finally, BUT. Based on this information, DMBG could be successfully used as an internal standard in the determination of 2-AI and BUT at their respective optimum detection conditions. Subsequently, 2-AI determination was carried out using phosphate separation BGE at 190 nm, while BUT determination was carried out using formate BGE at 240 nm. To simulate the conditions in samples related to 2-AI and BUT consumption, collected human saliva was spiked with the respective analyte (10 and 20 μM) in the typical concentration range reported in the literature for members of the SC family[12–14] At this stage, DMBG (53.3 μM) was also added to the saliva to serve as an internal standard. Afterward, sample preparation was carried out. An exemplary electropherogram of 2-AI (at 190 nm, left) in saliva at 10 μM (measured concentration: 7.5 μM after sample preparation) and BUT (at 240 nm, right) in saliva at 10 μM (measured concentration: 7.5 μM after sample preparation) is shown in Fig. 5. Determinations of 2-AI and BUT using DMBG

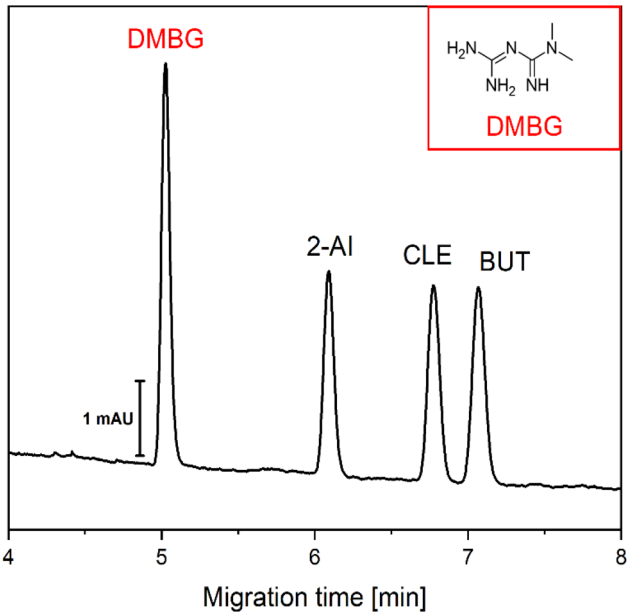

Fig. 4 CE-UV measurements for the test of dimethylbiguanidine as an internal standard. Electropherogram for a mixture of 100 μM 2-AI, BUT, CLE, and 100 μM DMBG in phosphate BGE (100 mM sodium dihydrogen phosphate and 100 mM phosphoric acid). UV detection at 200 nm. Structure of DMBG right upper corner (red). CE conditions: separation voltage 15 kV, capillary ID 50 μm, capillary length 60 cm (effective length 50 cm). The injection was controlled by a siphon effect driven by a height difference of 10 cm for 15 s.

Table 3 Concentrations of 2-AI and BUT before (*C* (sal.)) and after sample preparation (*C* (treated)) in human saliva samples, measured concentrations using the method of internal standard, and recovery (Rec.) for saliva samples spiked with 10 and 20 μM 2-AI and BUT. Measurements at 190 and 240 nm ($n = 3$)

Species	*C* (sal.) [μM]	*C* (treated) [μM]	*C* (measured) [μM]	Rec. [%]
Phosphate BGE 190 nm				
2-AI	10.0	7.5	7.37 ± 0.03	98
	20.0	15.0	15.0 ± 0.5	100
Formate BGE 240 nm				
BUT	10.0	7.5	7.6 ± 0.1	102
	20.0	15.0	15.1 ± 0.5	101

as an internal standard at their optimum conditions are summarized in Table 3.

Quantitation of 2-AI and BUT at optimum conditions resulted in a concentration of 7.4 ± 0.03 and 15.0 ± 0.5 μM for 2-AI and a concentration of 7.6 ± 0.1 and 15.1 ± 0.1 μM for BUT after sample preparation. The experiments showed a high recovery in the range of 98–102% for both 2-AI and BUT at both concentrations investigated. This leads to the conclusion that the developed CE-UV method, in combination with minimal sample preparation, is suitable for 2-AI and BUT determination in the forensic context. Analogous to BUT and 2-AI, CLE and other members of the SC family could be determined at their

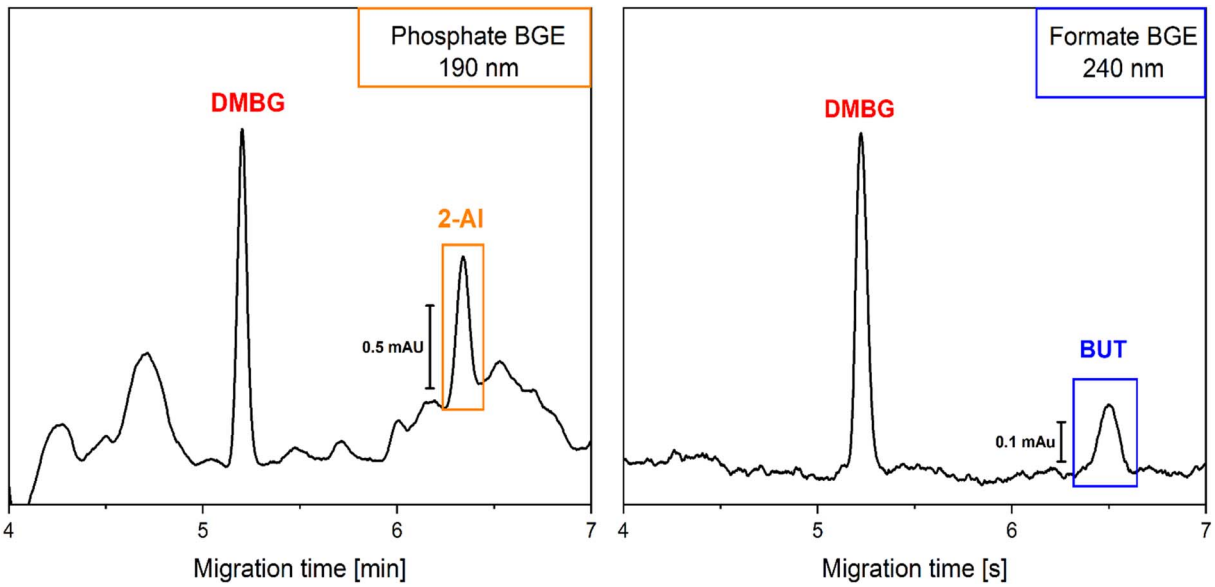

Fig. 5 Determination of 2-AI and BUT in human saliva at forensic concentration levels (human saliva was spiked with 10 μM (20 μM) of analyte before sample treatment). Left: Electropherogram of 2-AI (7.5 μM) in human saliva after sample preparation using phosphate separation BGE (100 mM sodium dihydrogen phosphate and 100 mM phosphoric acid) and 40 μM DMBG as an internal standard (UV detection at 190 nm, optimum for 2-AI). Right: Electropherogram of BUT (7.5 μM) in human saliva after sample preparation using formate separation BGE (100 mM ammonium formate and 100 mM formic acid) and 40 μM DMBG as an internal standard (UV detection at 240 nm, optimum for BUT). CE conditions: separation voltage 15 kV, capillary ID 50 μm, capillary length 60 cm (effective length 50 cm). The injection is controlled by a siphon effect driven by a height difference of 10 cm for 15 s.

optimum conditions. In this study, DMBG was used as an exemplary internal standard, which was optimal for 2-AI and BUT determinations.

4 Conclusions

This study is intended to show the possibilities of determining substances from the SC family and aminoindane by using two prominent CE methods, namely CE-MS and CE-UV. We investigated the migration behavior and detectability of 2-AI, BUT, and CLE using formate and phosphate BGEs. Formate BGE was successfully used as a possible acidic-BGE in both CE-MS and CE-UV. Here, phosphate BGE emerged as the ideal BGE in CE-UV for acidic conditions, especially at detection wavelengths below 230 nm, as it showed no intrinsic UV absorption. With their high sensitivity and similar LODs in the lower μM range, CE-MS and CE-UV were suitable techniques for SC determination in the forensic context.

In this manuscript, we described a reliable, simple, and time and cost-efficient determination of 2-AI, BUT, and CLE in oral fluid utilizing a CE-UV setup. Based on this information, we were able to determine these substances in human saliva at concentration levels that have been reported in the context of substance abuse.[12–14] The developed protocols, including easy sample preparation, provide the possibility of directly using saliva samples without time-consuming extraction protocols and, therefore, a fast and cost-efficient way of drug monitoring for many clinical and forensic applications. However, these protocols are not limited to 2-AI, BUT, and CLE determination. Many species from the family of NPSs are commonly abused psychedelic substances. Prominent examples from this category, like methylone, mephedrone, flephedrone, or methylenedioxypyrovalerone (MDPV), could be considered. For example, between 2009 and 2011 in the United Kingdom, approximately 60 verified fatalities were associated with the consumption of mephedrone,[26] and it continues to be widely available in the drug market today. Hence, methods for its quick and easy detection need to be available. Furthermore, in CE-UV, a diode-array detector could be used instead of a single-wavelength device, whereby two or more analytes could be quantified at their individual optimum detection wavelength in one measurement run.

Data availability

The data supporting this article have been included as part of the ESI.†

Author contributions

Martin Koall: conceptualization, data analysis, methodology, investigation (CE-UV, CE-MS), writing (manuscript). Eva Pospisilova: conceptualization, investigation (CE-UV), writing (introduction), Joachim Rewitzer: investigation (photometry, CE-UV), Thomas Herl: review, Tatiana V. Shishkanova: provision of analytes, review, Frank-Michael Matysik: conceptualization, supervision, review.

Conflicts of interest

There are no conflicts to declare.

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