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A rapid and simple method for the determination of psychoactive alkaloids by CE-UV: application to *Peganum Harmala* seed infusions

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The β-carboline alkaloids of the harmala (HAlks) group are compounds widely spread in many natural sources, but found at relatively high levels in some specific plants like *Peganum harmala* (*Syrian rue*) or *Banisteriopsis caapi*. HAlks are a reversible Mono Amino Oxidase type A Inhibitor (MAOI) and, as a consequence, these plants or their extracts can be used to produce psychotropic effects when are combined with psychotropic drugs based on amino groups. Since the occurrence and the levels of the HAlks in natural sources are subject to significant variability, more widespread use is not clinical but recreational or ritual, for example *B. caapi* is a known part of the Ayahuasca ritual mixture. The lack of simple methods to control the variable levels of these compounds in natural sources restricts the possibilities to dose in strict quantities and, as a consequence, limits its use with pharmacological or clinical purposes. In this work, we present a fast, simple, and robust method of quantifying simultaneously the six HAlks more frequently found in plants, i.e., harmine, harmaline, harmol, harmalol, harmane, and norharmane, by capillary electrophoresis instruments equipped with the more common detector UV. The method is applied to analyze these HAlks in *P. Harmala* seeds infusion which is a frequent intake form for these HAlks. The method is validated in three different instruments in order to evaluate the transferability and to compare the performances between them. In this case, harmaline, harmine, and harmol were found in the infusion samples. Copyright © 2016 John Wiley & Sons, Ltd.

Keywords: capillary electrophoresis; β-carboline, harmala alkaloids; optimization; Peganum harmala

Introduction

Harmala alkaloids (HAlks) are a family of compounds which have known effects as reversible inhibitors of the Mono Amino Oxidase (MAO) enzymes. Some of the members have selective inhibition of the MAO Type A (MAOI-A) and some others inhibit the MAO Type-B (MAOI-B).[1] Therefore, these compounds, administered alone or in combination with other drugs, can produce a variety of psychotropic effects. Even though these effects could be used with pharmacological/clinical objectives, these plants are nowadays used exclusively to prepare ritual preparations such as ayahuasca, yage, huanto, and other decoctions, or recreational drug cocktails. Others effects such as sedative and anxiolytic effects,[2] antibiotic activity,[3] and induction of lung cancer cells death via autophagy[4] have been reported, conferring on the natural sources of these compounds enormous potential to be used in clinical or pharmacological objectives. HAlks are widely spread in nature, principally in plants, and nowadays can be detected at low levels in common foods and consumer products. For example, they are present in plants of the *Passiflora* family such as passion fruit (*Passiflora edulis*) and its derived products,[5] in cocoa and chocolate,[6] in coffee,[7] in tobacco smoke,[8] and in other sources.

In these cases HAlks are present at levels below the required quantities to produce noticeable effects in human beings. However, there are specific plants where HAlks can be found at high concentrations.[9–14] For example, harmine and harmaline are present in *Banisteriopsis caapi* or *Peganum harmala* (Syrian rue), which are known components of the ayahuasca, yage, and huanto and other ritual decoctions used in typical religious practices. In general, these infusions consist of a mixture of an herb containing high levels of HAlks with another providing a tryptamine.[9–14]

Estimations of the maximum daily intake have been reported,[15] although, no regulation has been established for HAlks or for the plants containing them.

In order to obtain advances in the clinical/pharmacological use of HAlks and their natural sources over their use as a recreational or ritual drug, analytical tools must be provided allowing more studies of

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their occurrence in nature, their physical distribution, their different life stages and/or growing conditions, metabolic pathways in humans and elimination, etc. In In this context, new methods to determine HAlks in natural sources are highly valued. Given that pharmacologically active compounds occur at relatively high concentrations, the analytical exigencies do not aim to improve the limits of detection (LODs), but rather other analytical figures, such as efficiency and resolution, or analysis time. High efficiencies gives more chances to successfully apply the same method to a variety of plants/sources/matrices. Optimized separation conditions translate into optimum resolutions which, at the same time, allows simultaneous exploration or quantification of many compounds in the same run. Other characteristics of the methods such as short analysis time, low cost, or the possibility of using autosamplers, allow to do wide screenings of the desired components in nature, on a high number and variety of natural plants and sources. Simple methods based on standard instruments usually result more robusts, which leads to improved transferabilities and also to wider accessibility of the method.

In the specific case of HAlks, the separation of the different members of the family is a challenging task, principally because of their similarities.[16–18] They are all based on the same β-carboline structure (9H- pyrido [3, 4-b] indole), changing only one substituent and/or an insaturation, without significant molecular mass differences (Figure 1). Furthermore, they present at least one amino ionizable group and they are sparingly soluble in water. High performance liquid chromatography (HPLC) coupled to different detection systems like UV, fluorescence, and mass spectrometry (MS), is the usual technique for determining HAlks in different natural sources. Reports on HPLC determination of HAlks can be found for plants, foods, beverages, tobacco, or biological fluids.[6,7,11,13,19–23] The main issues of these LC analyses are the relatively long time of analysis, and the operative cost. This is due to (1) the use of considerable amounts of solvents and (2) the variable but finite lifetime of columns and/or pre-columns when it is used to analyze real samples; i.e., capillaries must also be changed from time to time, although its cost is negligible in comparison with HPLC columns and pre-columns.

In the case of non-volatile analytes, capillary electrophoresis (CE) also offers a large list of advantages with respect to LC,[21] including improved efficiencies typically 10-fold higher with respect to LC; shorter analysis times; lower requirement of reagents and solvent; because of the very low cost of the capillaries, no cares or worries regarding risking the separation media, etc. Since HAlks are ionizable compounds, CE methods have been certainly explored by a few authors, either using the free capillary zone electrophoresis mode (CZE) and also using micellar electrokinetic chromatography (MEKC).[22,24–27] These authors have demonstrated the advantages of CE technique for the analysis of HAlks at high concentration in standard mixtures and plant extracts. However, the optimization of variables used in these cases allowed only to achieve baseline separation of few HAlks, with limited resolution and requiring very long analysis times.[28] The method was applied to determine low amounts of harmine, harmaline, and harmalol in wakame (*U. pinnatifida*), an edible marine invasive brown algae originally from East Asian countries that is commonly used as a foodstuff, as well as by traditional oriental medicine practitioners. However, despite the advantages of MS detection, on the one hand the requirements imposed by the ESI ionization to the background electrolyte, i.e., volatility, restrict the complete baseline resolution between all the members of HAlks family. However, this issue can be certainly compensated by using extracted ion electropherograms (EIE). Moreover, the variety of configurations and CE-MS interfaces make complex the method transfer between different instruments. On the other hand, the operational cost of MS detectors remains very expensive compared to ultraviolet (UV) detection for routine analysis, which is the built-in detection system typically available in all commercial CE instruments, even in CE-MS instruments.

In this work, we develop, optimize, and validate a method to analyze a mixture of six HAlks (i.e., harmine, harmaline, harmol, harmalol, harmane, and norharmane) by CE-UV. Background electrolyte (BGE) pH, ionic strength, organic solvent composition, and separation voltage were optimized to achieve a fast and complete baseline separation. The optimized method was validated in terms of linearity, limits of detection and quantification, repeatability and reproducibility. Furthermore, inter-instrumental method transfer was evaluated for three different commercial instruments. The method was applied to determination of HAlks in an infusion prepared from the psychoactive seeds of *P. Harmala*.

Materials and methods

Instrumentation

Experiments were performed on a CE Lumex Capel 105 M (with UV detector), (Lumex Ltd, St Petersburg, Russia), a CE system P/ACE MDQ (with DAD detector) (Beckman Coulter, Brea, CA, USA) and an Agilent Technologies HP^{3D}CE (with DAD detector) (Waldbronn, Germany). pH measurements were performed with a Crison 2002 potentiometer (Crison Instruments, Barcelona, Spain) and a Schott

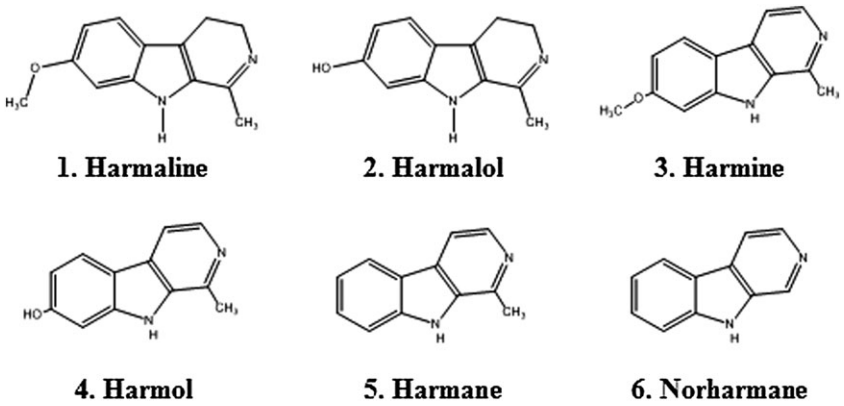

Figure 1. Chemical structure of the studied harmala alkaloids (HAlks). Monoisotopic molecular mass (M): (1) harmaline 214.27 pKa: 9.55, (2) harmalol 200.24 pKa: 8.62 and 11.3, (3) harmine 212.25 pKa: 7.55 (4) harmol 198.22 pKa: 7.45 and 9.52 (5) harmane 182.22 pKa: 7.34 (6) norharmane 168.19 pKa: 6.80.

Blueline 11-pH glass combination electrode (SI Analytics GmbH, Mainz, Germany). Sample incubation was performed with a Thermo-Shaker TS-16 100 (Biosan, Michigan, USA). Centrifugation was carried out in a thermostated Rotanta 460 centrifuge (Hettich Zentrifugen, Tuttlingen, Germany).

Chemicals

Solutions were prepared with water provided by a MilliQ® water purification system (Millipore, Bedford, MA, USA). Methanol was HPLC grade (Merck, Darmstadt, Germany). The rest of chemical reagents were analytical grade or better. Ammonia was purchased from Carlo Erba Reagents S. A. (Sabadell, Spain), *tris* was from J. T. Baker chemicals (Serviquimia, Barcelona, Spain), and hydrochloride acid, acetic acid and phosphoric acid were from Merck (Darmstadt, Germany). Harmane, norharmane, harmalol hydrochloride dihydrate, harmol hydrochloride dihydrate, harmine, and harmaline were purchased from Aldrich (Steinheim, Germany). An individual stock standard solution of each alkaloid was prepared by dissolving the solid in methanol at a concentration of 100 μg/mL. All the stock solutions were stored at 4 °C. The working solutions were obtained by dilution with water and filtered through 0.22 μm nylon membrane before use.

Procedures

Electrophoretic conditions

All the analyses were performed with a 60 cm total length (L_T) and 75 μm internal diameter (i.d.) fused-silica capillary supplied by Polymicro Technologies (Phoenix, AZ, USA) at a temperature of 25 °C. Under optimized separation conditions the BGE was 50 mM *tris*-HCl (pH 7.8) with 20% (*v/v*) of methanol. All capillary rinses were performed at high pressure (i.e., 930 mbar in HP^{3D}CE instrument). New capillaries were activated by flushing with 1 M NaOH (20 min), water (15 min), and BGE (30 min). The capillary was finally equilibrated by applying the separation voltage for 15 min (20 kV). Between runs, the capillary was rinsed with 0.1 M NaOH (1 min), water (1 min), and BGE (3 min). Samples were hydrodynamically introduced into the capillary at 35 mbar for 3 s and the separation was conducted under normal polarity (cathode at the outlet) by applying a voltage of 20 kV. The UV window was placed at 50, 51, and 52.5 cm from the inlet of the capillary (L_D) for P/ACE MDQ, Capel 105 M and HP^{3D} CE instruments, respectively. It must be pointed out that the L_T was kept constant between instruments, despite the differences in L_D, in order to maintain the electric field constant during the separations. Considering the absorption UV spectra reported by Cheng and Mitchelson,[24] the detection was performed at 200 nm and UV-lamps were in optimum working conditions.

Sample pretreatment of P. harmala

P. harmala seeds were purchased in a so-called grow shop in Barcelona (Spain). The seeds (10 g) were milled in an electric coffee mill. Infusions were prepared using 0.5 g of this powder.[12–14] Ten mL of water was added and heated gently with a Bunsen burner until boiling started. Then, the mixture was centrifuged for 10 min at 8000 G and the supernatant was collected. The process was repeated two more times and all the supernatants were pooled together. This ~30 mL of aqueous extract/infusion was raised to 50 mL of final volume with water. This stock sample solution was filtered with 0.22 μm nylon filter before diluting 1:250 *v/v* with BGE for the analysis.

Results and discussion

CE-UV method optimization and validation

The optimization of the CE-UV separation consists of several steps. Since HAlks are ionizable compounds (Figure 1), the pH is the main variable to investigate. In a previous study,[29] we described a method to optimize the electrophoretic separation of ionizable compounds. The separation is given in terms of mobility differences and, consequently, the method involves the hypothesis that peak widths (and resolutions) can be optimized on a lately independent procedure. The consideration of pH as an optimization variable requires, first, to describe the effective mobility of each compound as a function of the mobility of the fully ionized form (actual mobility) multiplied by its ionization degree, which is defined by its dissociation constant and the pH of the BGE. Then, a single mathematical function to qualify the whole separation (MultiCriterion Optimization Function, MCOF) can be composed on the basis of considering simultaneously (1) the separation between compounds by pairs, and (2) the separation of each compound from the neutral compounds. The optimum pH values can be obtained by mathematical maximization of the single function, MCOF. Finally, an empirical optimization of other variables, such as type of buffering compound or solvent used as additives, can lead to the optimized conditions where the best possible separation can be achieved. When applying the aforementioned procedure to our case of study, a function qualifying the separation of the six HAlks can be obtained, and its maximization leads to an optimum value pH = 7.8. When running at this pH value, some analytes will be partially ionized and, therefore, the migration order does not follow an order based on their molecular sizes/masses, but also and principally on the differences in the ionization degrees. A rough but better approach could be done for monoprotic analytes, analyzing the differences between the pKa and the optimum pH. However, this exceeds the aim of this work and further details on these fundamental aspects can be found in a separate work.[29]

The advantages of using different compounds as a background electrolyte was also evaluated by analyzing the mixture of HAlks at the optimum pH value, but using phosphate, ammonium acetate, or *tris*, at the same ionic strength (I = 50 mM). Results are shown comparatively in Figure 2.

As noted, the use of *tris* buffer (Figure 2C) produces the best compromise between peak height, efficiency, resolution, and total analysis time, although peak shapes are not satisfactory for HAlks number 3 and 4.

Other ionic strength levels (10–100 mM) have been also evaluated without significant improvements.

Finally, the addition of small proportions of acetonitrile or methanol in the solvent of the 50 mM *tris* BGE solution was evaluated.

The addition of organic solvents can affect the dielectric constant (ε), polarity (μ), viscosity (η), and solubilities. As a consequence, a known effect associated with the use of solvents is to have lower operating currents and lower Joule heating, which usually leads to an improvement of the peak widths and in the efficiency.[30] The addition of organic solvents could affect the acid-base properties and the ionization degree of some of the analytes; however, the use of low percentages of the more polar solvents (0–20% by volume of acetonitrile or methanol) do not change it significantly when the ionizable group of buffer and analytes are of the same type while, in practice, these small quantities of solvent use to be enough to improve the peak shapes, peak widths and separation

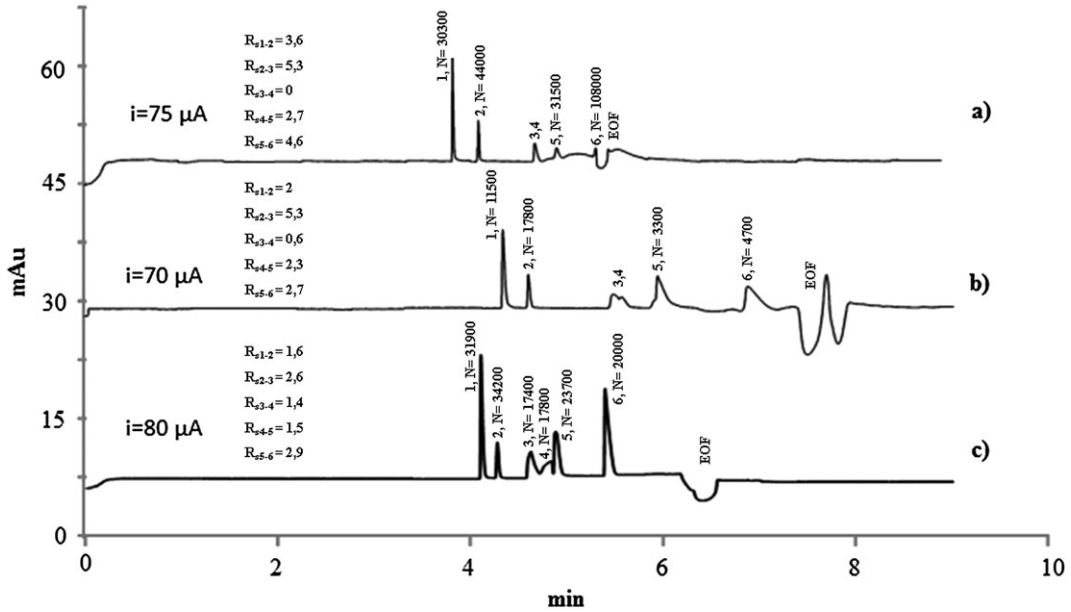

Figure 2. CE-UV separation (254 nm) of (1) harmaline, (2) harmalol, (3) harmine, (4) harmol, (5) harmane, and (6) norharmane at a concentration of 10 µg/mL. Voltage was 20 kV. Instrument was Capel 105 M. The separation was performed with different BGE at pH 7.8 and 50 mM ionic strength, (A) NaH_2PO_4/Na_2HPO_4, (B) ammonium acetate, and (C) *tris*. (i = current intensity). R_s represents the resolution and N the efficiency.

efficiencies. In this study, acetonitrile and methanol were evaluated but the last one lead to better results in terms of peak shapes and peakwidths.

The quantity of methanol added to the solvent based on the 50 mM *tris* (pH 7.8) BGE is shown in Figure 3. The 50 mM *tris* (pH = 7.8) in methanol 20% BGE provided the best peak shape, improving efficiencies and achieving baseline resolution of the six HAlks in less than 10 min.

Using this optimized BGE, resulting peak capacities were in general excellent, and the number of theoretical plates (calculated as $N = 5.54\ (t_m/w_{1/2})$) ranged from 85000 for harmol to 335000 for harmaline (peaks 4 and 1 respectively on Figure 3c). Complete baseline resolution ($R_s = 1.5$) is achieved between the critical pair of analytes (peaks 3–4, Figure 3C, harmine–harmol), while Rs for any other pair is higher than 2.5.

In contrast with our results, in other reports based on HPLC or CE methods, the total analysis time are always longer than 15 min, but in any case baseline resolution of the six alkaloids is achieved.[11,19,22,24–27] The resolution of the critical pair (3–4, Figure 3C, harmine–harmol) was complete ($R_s = 1.5$) and between the rest of the pairs the R_s was higher than 2.5.

The analytical figures of merit obtained in three different commercial CE instruments were calculated from experimental data by measuring peak area and migration times of the HAlks. Studies of repeatability (intra-day, n = 10) and reproducibility (inter-day, three different capillaries on three different days, n = 10) were performed by analyzing a mixture of the six HAlks at a concentration of 25 µg/mL. These parameters were calculated as a percentage of the RSD (RSD %) based on peak areas and migration times. The limits of detection (LODs) and limits of quantitation (LOQs) for each of the

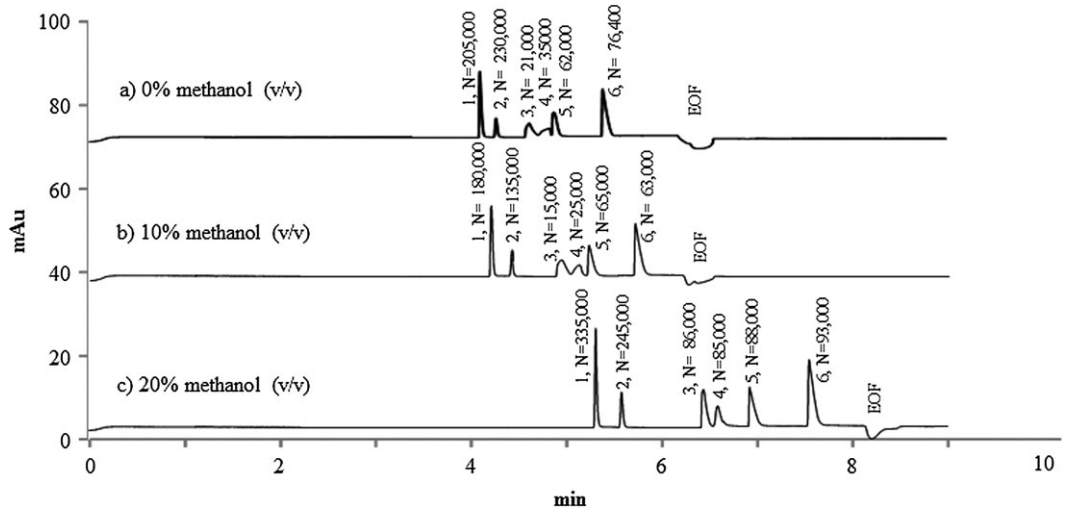

Figure 3. CE-UV separation (254 nm) of (1) harmaline, (2) harmalol, (3) harmine, (4) harmol, (5) harmane, and (6) norharmane at a concentration of 10 µg/mL. Voltage was 20 kV. Instrument was Capel 105 M. Separation was performed with a 50 mM *tris* BGE at pH 7.8 with different concentrations of methanol (A) 0 %, (B) 10 %, and (C) 20% (*v/v*). (N = number of theoretical plates).

HAlks were calculated as three times and ten times the signal-to-noise ratio (S/N), respectively. Linearity was evaluated in the concentration range of 5–120 μg/mL. Calibrations were done at seven concentration levels: 5, 10, 15, 25, 50, 100, and 120 μg/mL, and analyzing duplicates or triplicates at each level. For HAlk quantification, three different infusion samples were analyzed by triplicate, and the concentrations were calculated from the external standard calibration curves.

Once the separation was optimized, the method was validated in terms of linearity, LOD, and LOQ with three different commercial instruments at 200 and 254 nm (Capel 105 M, HP^{3D}CE, and P/ACE MDQ). As an example, the figures of merit at 254 nm, which is the wavelength typically reported in the literature to analyze compounds with aromatic rings free of groups producing shifts, are summarized in Table 1. As can be observed, linearity range is the widest with Capel 105 M instrument (0.03–120 vs 0.1–70 μg/mL, $R^2 > 0.99$), which also allows, in general, the lowest LODs and LOQs (between 0.01 and 0.12 μg/mL and 0.03 and 0.4 μg/mL, respectively) as expected when comparing photomultiplier (UV) with photodiode array detectors (DAD). LODs and LOQs are approximately twice as high with a P/ACE MDQ instrument, which is especially sensitive, as indicated by the large values of the calibration curve slopes. HP^{3D}CE provided LODs and LOQs between 15 and 30 times higher than the other instruments and has the lowest sensitivity. It is could be attributed to the small section of light beam collected in the few microscopic diodes of the DAD chip, and also to the use of a simple slit to restrict the light beam passing through the lumen of the capillary tube. The other designs are based on the use of spheric lenses to focus the light beams from a greater cross section into the lumen of the capillary tube to, finally, detect the intensity not with a small section chip but with standard size phototube/photodiode. Repeatability on migration time and peak areas are similar between the different instruments. Table 1 shows the repeatability values of migration times and peak areas for the three instruments, which ranged between 1.2–1.5% and 2.4–8.3% for the Capel 105 M, between 0.8–1.4% and 2.2–6.5% for the HP^{3D}CE, and between 0.9–1.6% and 3.3–8.2% for the P/ACE MDQ, respectively. Reproducibility is also very similar between them, with values for migration time and peak area ranging between 1.6–2.5% and 3.5–11.1% for Capel 105 M, 1.5–2.1% and 4.2–8.9% for HP^{3D}CE and 1.9–3.2% and 5.5–11.6% for P/ACE MDQ, respectively (data not shown in Table 1). As expected reproducibility is slightly lower than repeatability (RSD % slightly higher), but these values are typically found in CE-UV methods using organic solvent as additives in the BGE.

Table 1. Linear range, linear regression, limits of detection (LODs), limits of quantification (LOQs) and repeatability of migration times and peak areas (%RSD = relative standard deviation) for the six HAlks using the optimized CE-UV method in three different CE instruments at 254 nm (standards at 25 μg/mL, n = 10)

HAlk	Instrument																	
	Lumex capel 105M						Agilent HPCE3D						Beckman P/ACE MDQ					
	Linear range (μg/mL) (R^2>0.99)	Linear regression (Y=a*C+b)	LOD (μg/mL)	LOQ (μg/mL)	RSD % time (n=10)	RSD % Area (n=10)	Linear range (μg/mL) (R^2>0.99)	Linear regression (Y=a*C+b)	LOD (μg/mL)	LOQ (μg/mL)	RSD % time (n=10)	RSD % Area (n=10)	Linear range (μg/mL) (R^2>0.99)	Linear regression (Y=a*C+b)	LOD (μg/mL)	LOQ (μg/mL)	RSD % time (n=10)	RSD % Area (n=10)
Harmaline	0.2-120	1.4*C+2.1	0.06	0.2	1.2	8.2	4.8-70	0.4*C-1	1.2	4.8	1.2	6.5	0.6-70	262*C-80	0.2	0.6	1.3	8.2
Harmalol	0.4-120	0.8*C+1.64	0.1	0.4	1.2	2.4	8.3-70	0.2*C+0.3	2.1	8.3	1.2	2.2	1.1-70	142*C-27	0.3	1.1	1.3	3.3
Harmine	0.05-120	5.7*C+3.2	0.02	0.05	1.4	8.3	1.2-70	1.7*C+1.2	0.3	1.2	1.4	6.3	0.1-70	1705*C-200	0.03	0.1	1.6	7.5
Harmol	0.1-120	4.7*C-0.8	0.02	0.1	1.4	6.9	1.3-70	1.5*C-3.2	0.3	1.3	1.2	5.3	0.1-70	1428*C-286	0.03	0.1	1.3	4.9
Harmane	0.05-120	6.1*C+3.5	0.01	0.05	1.4	5.2	1.1-70	1.8*C-0.7	0.3	1.1	1.0	5.4	0.1-70	1891*C-20	0.02	0.1	0.9	5.4
Norharmane	0.03-120	10.9*C+3.8	0.01	0.03	1.5	2.4	0.7-70	2.8*C-2	0.2	0.7	0.8	3.5	0.1-70	2746*C-80	0.02	0.1	1.1	5.1

Analysis of P. harmala

The method was applied to an infusion prepared from the seeds of *P. harmala*. Figure 4 shows the CE-UV electropherograms of the diluted infusion (1:250) at 200 nm and 254 nm with the HP^{3D}CE instrument. At 200 nm (Figure 4A), harmaline (1), harmine (3), and harmol (4) were identified by comparison analyses of infusion samples and infusion samples spiked with standards. Statistic comparison of slopes obtained by means of the standard additions methods with the obtained in the external calibration methods did not demonstrate significant differences, discarding any matrix effect. Additionally, in separate experiments by CE-MS peak purity criteria have been applied proving that no other compounds co-migrate with any of the HAlks. At 254 nm (Figure 4B) the background signal due to the UV absorbing matrix interferences was high and only harmine could be detected. At 200 nm (Figure 4A), in addition to the three HAlks, an abundant component was also

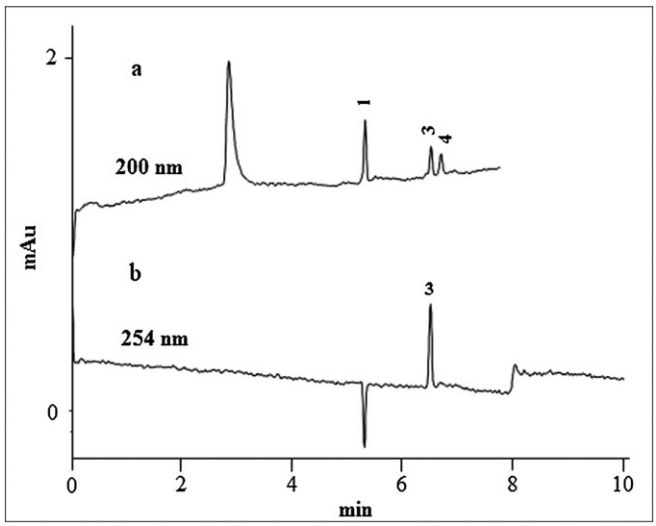

Figure 4. Separation of (1) harmaline, (2) harmalol, (3) harmine, (4) harmol, (5) harmane and (6) norharmane in the diluted *P. harmala infusion* (1:250) at: (A) 200 nm and (B) 254 nm. The separation voltage was 20 KV. Instrument was $HP^{3D}CE$. The BGE was 50 mM *tris* BGE at pH 7.8 with a methanol concentration of 20 % (*v/v*).

detected at around 3 min, which according to Frison *et al.*[14] could be tentatively identified as peganine, a quinazoline alkaloid.[14,27] An external standard calibration method using 200 nm was used to quantify the detected HAlks. Excellent linearity was observed with the range 4–100 μg/mL, $R^2 > 0.99$. The results of the calibration curves were S = .5.1*C + 2.9, LOD: 1.1 μg/mL and LOQ: 3.8 μg/mL for harmaline, S = 4.8*C + 2.8 and LOD: 1.2 μg/mL and LOQ: 4.0 μg/mL for harmine and S = 5.5*C + 0.16 and LOD: 0.06 μg/mL and LOQ: 0.2 μg/mL for harmol. Repeatability and reproducibility of areas at 200 nm were, 1.5% and 8%, 1.4% and 8.2%, and 1.2% and 7%, for harmaline, harmine, and harmol, respectively. Harmaline, harmine, and harmol were present in undiluted infusion samples, and seeds at concentrations of 53 (±2), 24.3 (±0.4), and 16.4 (±0.7) μg/mL and 0.32 (±0.01), 0.151 (±0.004), and 0.098 (±0.004) % (w/w), respectively. These values, and the harmine/harmaline ratio (0.46), are in agreement with those that can be found in the literature[12–14,31] taking into account that the amounts of alkaloids in a infusion prepared from water decoction or infusion are supposed to be substantially different than in organic extracts. Furthermore, the total and relative amounts of the different alkaloids in the different plant materials change at different stages of growth, climate, or geographical origin. It is widely accepted that the total alkaloid content in *P. harmala* seeds vary between 2 and 7% (w/w).[12–14] However, there is little information about characterization of infusions prepared from seed decoction or infusion. G. Frison *et al.* found in a *P. harmala* seed organic extract a content of 12.00 and 7.20 mg/mL of harmaline and harmine, respectively.[14] Ayahuasca beverages, which are also prepared as infusions by water decoctions, have been characterized to a large extent, and values ranging from 0.003 to 0.2 and from 0.07 to 1.70 μg/mL has been reported for harmine and harmaline, respectively.[10,14,32] In contrast to harmaline and harmine, for harmol there is no consensus on the concentration range in P. harmala and while some authors have found it as a minor component, some others found it as a major one. It could be related with the fact that harmol is the main metabolite of harmine and an easy conversion of one into the other can be affecting the consistency of the results found by different authors.

Conclusions

A novel and fast method to quantify the six most frequently found HAlks (i.e., harmine, harmaline, harmol, harmalol, harmane, and norharmane) by CE-UV in less than 10 min was developed. It was proved that the addition of methanol in the BGE significantly improves peak shape, efficiency, and resolution, without changing selectivity. Optimum separation in fused silica capillaries was achieved using a BGE of 50 mM *tris*-HCl (pH 7.8) with 20% (*v/v*) of methanol. The method was validated and, repeatability, reproducibility, and method transfer between three different commercial instruments evaluated. The method demonstrated to be very robust in the instruments used in this work. It basically addresses the simplicity of the BGE and the selectivity of a specific pH condition. In practice the Lumex Capel 105 M allowed detection of these HAlks at the lowest levels, which could be attributed to the differences in the optical configurations of the detectors. The method was successfully applied to P. harmala seeds infusions which have relatively high concentrations of harmine, harmaline, and harmol.

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