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Electric field-assisted multiphase extraction to increase selectivity and sensitivity in liquid chromatography-mass spectrometry and paper spray mass spectrometry

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ABSTRACT

In this work, for the first time, chromatographic paper was used for a multiphase extraction assisted by an electric field (MPEF) and directly coupled to paper spray mass spectrometry (PS-MS). Using this approach, five tricyclic antidepressants (TCAs) were determined in oral fluid. Firstly, the MPEF conditions were optimized using liquid chromatography-mass spectrometry (LC-MS/MS). The effects of the chromatographic paper and the types of electrolyte used in the acceptor phase, the organic solvent type and the amount used in the donor phase, the extraction time, and the applied electric potential were all investigated. After optimization, the analytes were extracted from the donor solution (sample and acetonitrile 1:1 (v/v)) over a period of 10 min at 300 V, crossing the free liquid membrane (1-octanol) and reaching the acceptor phase (chromatographic paper wetted with 400 mmol L^{-1} acetic acid). The method using LC-MS/MS was validated, demonstrating a linear range from 2 to 12 ng mL^{-1}, with detection and quantification limits of 0.13–0.25 and 0.44–0.84 ng mL^{-1}, respectively, an intraday precision of less than 20%, and no matrix effect observed. The optimized MPEF conditions were then applied to determine TCAs by PS-MS and for this analysis cyclobenzaprine was used as an internal standard. The easy, fast and direct approach of coupling MPEF with PS-MS analysis, as well as the pre-concentration and the low standard deviation of replicates (less than 20%), demonstrates that this method can be useful for screening in clinical and toxicological analysis.

1. Introduction

Direct analysis approaches have become increasingly more popular in recent years, and paper spray mass spectrometry (PS-MS) is one of the most widespread exponents within this field. The elegant strategy of ambient ionization for mass spectrometry analysis, presented by Cooks in 2009 [1], uses a small triangular piece of porous support (usually chromatographic paper with dimensions of 1.5 × 1.5 cm) to directly receive a small volume of sample, without any prior preparation. After sample application, a suitable solvent is dropped over the paper and the electric field assists the paper to form a spray with the analytes ionized at the entrance of the mass spectrometer. Several applications of PS-MS have already been reported for different analytes (drugs [2–7], pesticides [8–12], hormones [13], and peptides [14]) and samples (saliva [5], urine [3,15,16], plasma [2], blood [7,14,17], serum [17,18], beverages [6,8,12,19–22], environmental samples [9,23–25], and food [10,11,26–28]).

With any analytical strategy, from the established LC-MS to the new PS-MS approach, high amounts of salts, proteins, lipids, sugar, and other interferents make the analysis of biological fluids, food and environmental samples a singular challenge, especially if the number of runs per day is high. In the case of PS-MS, to overcome this difficulty, many authors have combined different sample preparation procedures, such as LLE (liquid-liquid extraction) [6], SPE (solid-phase extraction) [2], QuEChERS [12], protein precipitation [7], and so on.

Among recent approaches to sample preparation, electroextraction is one alternative that has been used with great success for the clean-up and pre-concentration of analytes present in complex matrices [29,30]. Electric fields can transfer charged analytes from a donor phase (sample) to an acceptor phase in a short time, with selectivity, efficiency, and a low consumption of solvent [31]. Some studies have described direct analysis by mass spectrometry after electroextraction without the need for chromatography or electrophoresis and the benefits of using fast and inexpensive techniques with focusing and selectivity

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capabilities, such as PS-MS, have been explored [32–38].

There are many strategies concerned with the use of an electric field in sample preparation, and the so-called multiphase extraction assisted by an electric field (MPEF), with a free liquid acceptor phase or one embedded inside a solid support, has attracted attention due to its robustness, versatility, and capability for the simultaneous extraction of multiple samples [33,39–41].

A recent study described the electroextraction of crystal violet (CV) from fish using a cellulose cone tip as a simultaneous porous support to the electrolyte solution and adsorbent to the analyte [40]. After extraction, the colored analyte was quantified by a digital image analysis method using a conventional flatbed scanner. The cellulose cone tip used in this work inspired us to use this porous support in PS-MS after the electroextraction as an easy and cheap technique to obtain more selective and clean extracts, as well as reliable analytical results.

In this study, this strategy was used for the first time to test five basic tricyclic antidepressants that are widely used around the world [42]. These basic drugs were extracted from oral fluid, a non-invasive sample that can be easily used for drug monitoring [43–46]. The porous support and extraction parameters were optimized by quantifying the drugs using liquid chromatography-mass spectrometry, and the concept of multiphase extraction assisted by an electric field coupled to paper spray mass spectrometry (MPEF-PS-MS) was then evaluated.

2. Experimental

2.1. Reagents, chemicals, and solutions

Acetonitrile (ACN) purchased from Merck (Germany), 1-octanol from Sigma Aldrich (U.S.A.) and methanol (MeOH) and isopropanol from J. T. Baker (U.S.A.) were all of liquid chromatography grade. Formic acid, hydrochloride acid, ammonium acetate were obtained from Merck (Germany). Acetic acid (HAc) was purchased from F. Maia (Brazil). Ethanol, calcium chloride dihydrate, carboxymethylcellulose sodium salt, sodium monohydrogen phosphate and CV were acquired from Synth (Brazil). Sodium chloride and α-amylase (*Aspergillus oryzae*) at 36 U mg^{-1} were obtained from Sigma Aldrich (U.S.A.). Potassium monohydrogen phosphate was acquired from Neon (Brazil). Potassium dihydrogen phosphate was purchased from Vetec (Brazil). All reagents used were of analytical grade and were used without prior purification. Deionized water (18.2 MΩ cm) was obtained from a Millipore high purity water dispenser (U.S.A). The chromatographic papers (1 Chr and 3 MM Chr grade) were purchased from Whatman (U.K.). The papers were cut into isosceles triangle tips (central angle of 50°) with a long rectangular body (~3.5 cm in length and 0.8 cm in width). The hydrochloride salts of doxepin (DOX), imipramine (IMI), amitriptyline (AMI), nortriptyline (NOR) and clomipramine (CLO), and the hydrochloride salt of cyclobenzaprine (CBZ), as an internal standard, were obtained from Sigma Aldrich (U.S.A.) (pK_a values are presented in Fig. S1). The stock solutions of these drugs at 500 μg mL^{-1} were prepared by dissolving each salt separately in methanol and keeping them under refrigeration at 4 °C.

2.2. Oral fluid samples

The synthetic oral fluid was prepared based on the work of Arain and co-workers [47], with the carboxymethylcellulose concentration adapted to 5 g L^{-1}. The human oral fluid samples were collected from ten healthy volunteers (five young women and five young men) by just dropping the fluid in a recipient at least 30 min after feeding or tooth brushing. The studies with human oral fluid were carried out according to the Declaration of Helsinki for studies on human subjects, following its approval by the Research Ethics Committee of the Federal University of Minas Gerais, Brazil (protocol number: CAEE 32011214600005149).

2.3. MPEF approach for LC-MS/MS and PS-MS

For extraction optimization and LC-MS/MS method validation, a free paper approach was used (Fig. 1(A) left), whereas the supported paper configuration (Fig. 1 (A) right) was chosen for the PS-MS analysis. A multiwell plate (Fig. 1 (B)), as previously described [40], was used during the extraction procedures. The perforated conductive inert metal originally described to support the cellulose cone tips [40] was adapted to the triangular chromatographic paper shape, as shown in Fig. 1 (C). During the extraction optimization, the TCAs at 0.5 μg mL^{-1} were extracted from 1 mL of the donor phase (saliva sample added acetonitrile 1:1), crossing the organic filter (750 μL of 1-octanol) and reaching the acceptor phase (1 Chr chromatographic paper wetted with HAc at 400 mmol L^{-1}). The electric potential was applied through an electrophoresis source (KASVI model K33–300 V, China) connected to a multimeter (Tekpower model TP4000ZC, U.S.A.) with a RS-232 communicator to measure the electric current and record it on a computer (Windows XP build version 07.12.05_1339).

2.4. Proof-of-concept of the multiwell electroextraction plate with the new approach using chromatographic paper

To evaluate the functionality of chromatographic paper as a sorbent in the acceptor phase, extractions with the cationic dye CV as a model compound were carried out. The donor phase consisted of 1 mL of CV at 2 μg mL^{-1} in a mixture of McIlvaine buffer pH 3.0 and acetonitrile (1:1, v/v). A volume of 750 μL of 1-octanol was used as an organic filter and the acceptor phase was chromatographic paper 3 MM Chr soaked with 70 μL of acetic acid (400 mmol L^{-1}). The extractions were carried out by applying 300 V for 3 min.

2.5. Electroextraction optimization

Univariate studies to optimize the electroextraction were carried out for the following parameters: organic solvent in the donor phase (acetonitrile, methanol or ethanol); electrolyte in the acceptor phase (acetic acid at 400 mmol L^{-1}, pH 2.50, formic acid at 400 mmol L^{-1}, pH 2.01 or HCl at 10 mmol L^{-1}, pH 2.01); solid porous electrolyte solution support (conventional chromatographic paper 1Chr used for PS-MS or 3 MM Chr). A multivariate study was carried out to optimize the extraction time, electric potential and percentage of organic solvent present in the donor phase through the Box-Behnken design. The levels used in this experimental design are shown in Table S1. The data were processed using Design Expert 11 software (Statease, U.S.A). For the LC-MS/MS analyses, after extraction, the analytes were desorbed from the paper with 25 μL of isopropanol dropped on both sides of the paper and then 500 μL of desorption solution (methanol:acetonitrile:acetic acid, 47.5:47.5:5, v/v/v) were added. The chromatographic paper and desorption solution were vortexed for 30 s and sonicated for an additional 10 min. In addition, the organic filter (diluted fivefold in acetonitrile) was analyzed by LC-MS/MS to verify the amount remaining in this phase. For the PS-MS analyses, the electroextraction with the supported paper configuration (Fig. 1 (A) right) was realized previously using an internal standard (cyclobenzaprine) in the donor phase and after TCAs were directly analyzed by the paper spray. All experiments were realized in triplicate.

2.6. LC-MS/MS and PS-MS conditions

The LC-MS/MS analyses were performed on an Acquity UPLC H-Class liquid chromatography system (Waters, U.S.A) coupled to a triple quadrupole mass spectrometer with an electrospray ionization source (Xevo TQD, Waters, Ireland). The chromatographic separation was performed using an Acquity UPLC HSS C18 column (1.8, 2.1 × 50 mm) (Waters, U.S.A). The oven and sample plate were kept at 45 and 25 °C, respectively. The mobile phase consisted of formic acid at 0.1% in water

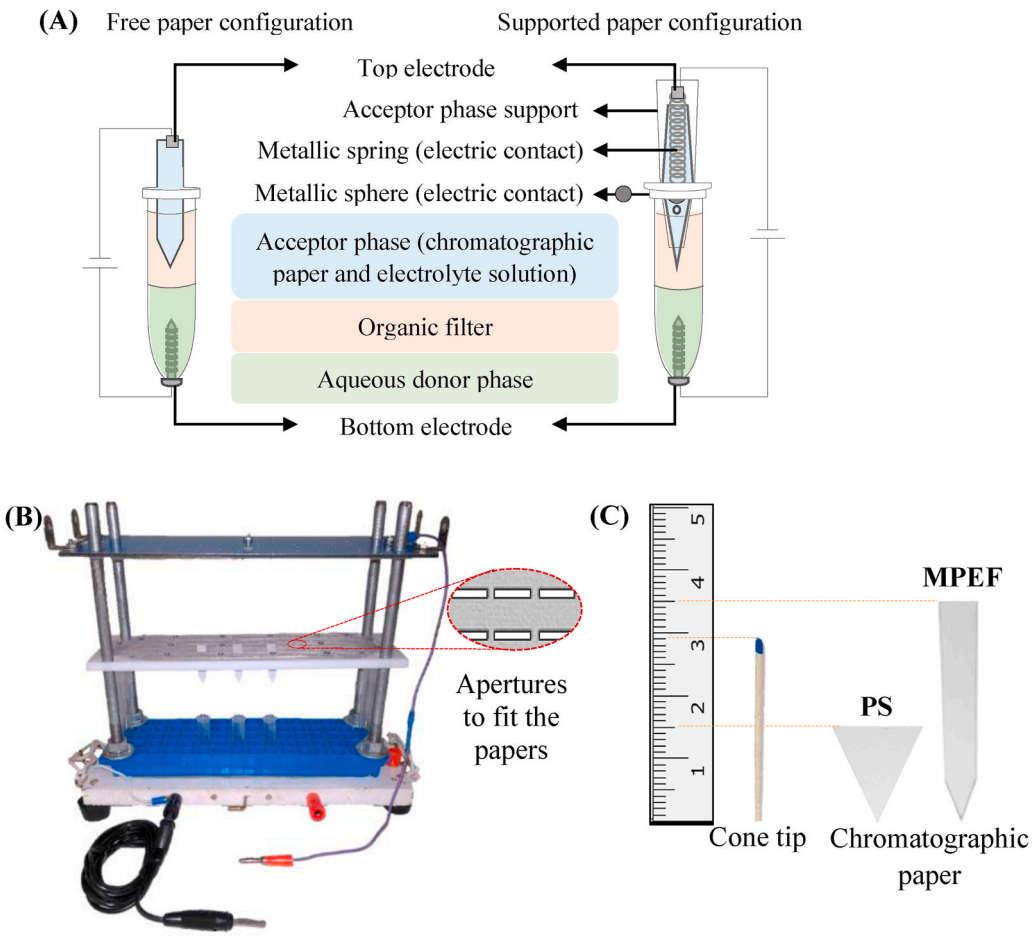

Fig. 1. Electroextraction schemes of the free paper (left) and the supported paper (right) configurations of the MPEF system, tube and paper (A). Multiphase multiwell plate for electroextraction (B) based on previous work [40] with adaptation for the chromatographic paper. Chromatographic paper used in the present work (MPEF), the usual chromatographic paper used for PS-MS direct analysis [1] and the cellulose cone tip for electroextraction describe elsewhere [39–47] (C).

(v/v) (A) and 0.1% in methanol (B) with a flow rate of 400 μL min^{-1} and the following gradient elution (time, %B): 0.00 min, 55%; 0.70 min, 95%; 1.70 min, 95%; 1.71 min, 55%; until 2 min, total time of analysis. An aliquot of 1 μL of the sample was injected into the LC-MS/MS system. The mass spectrometer was operated in positive ionization mode (ESI+) under the following conditions: source temperature, 150 °C; capillary voltage, 2 kV; desolvation temperature, 480 °C; desolvation gas flow, 720 L h^{-1}; cone gas flow, 20 L h^{-1}. The other conditions of the MS analysis are presented in Table 1.

The PS-MS analyses were performed on a LCQ Fleet mass spectrometer (Thermo Scientific, U.S.A.) with an electrospray ionization source and an ion trap analyzer. For this study, the electrospray ionization was replaced for a paper spray source, as shown in Fig. 2. The MS operating conditions were: capillary voltage, 43 V; capillary temperature, 275 °C; ionization voltage, +4 kV; tube lens voltage, 85 V; distance from paper tip to MS cone inlet, 5 mm; acquisition time, 0.2 min. For spray formation, a 50 μL solution of methanol:isopropanol: formic acid (95.45:4.45:0.1, v/v/v) was used. The m\z monitored were 264 (NOR), 278 (AMI), 276 (CBZ), 280 (DOX), 281 (IMI) and 315 (CLO).

Table 1
Transitions, collisions energy and cone voltages for TCA analysis by LC-MS/MS.

Analyte	Transitions (m/z)[a]	Collision energy (V)	Cone voltage (V)
DOX	280 → 107	26	40
	280 → 233	18	40
IMI	281 → 86	18	40
	281 → 58	30	30
AMI	278 → 91	26	40
	278 → 105	26	40
NOR	264 → 91	22	30
	264 → 105	22	30
CLO	315 → 86	18	40
	315 → 58	30	30

[a] The first transition was used for quantification and the second for confirmation.

2.7. Figures of merit

The analytical performance of the MPEF-LC-MS/MS method was evaluated based on the Eurachem guide [48]. The experiments were conducted using the synthetic oral fluid and applying 300 V for 10 min. The donor phase (sample of oral fluid), organic filter and acceptor phase consisted of: 1 mL mixture of sample:ACN 1:1 (v/v), 750 μL of 1-octanol, and chromatographic paper 3 MM Chr wetted with 70 μL of acetic acid (400 mmol L^{-1}), respectively. Linearity was investigated in the range 2–12 ng mL^{-1} with six points evenly spaced and three replicates (n = 3) for each concentration level. The matrix effect was investigated comparing three calibrations curves prepared in the desorption solvent and the synthetic and human oral fluid extracts. The slope and linear coefficient of the curves were compared through statistical F- and t-tests at a 95% confidence level. The limits of quantification (LOQ) and detection (LOD) were assessed using ten blank extracts fortified with 0.5 ng mL^{-1} TCAs. These parameters were estimated using $LOD = 3s_0/n^{1/2}$ and $LOQ = 10s_0/n^{1/2}$, where s_0 is the standard deviation of the TCA concentration and n is the number of replicates, in this case 10. The

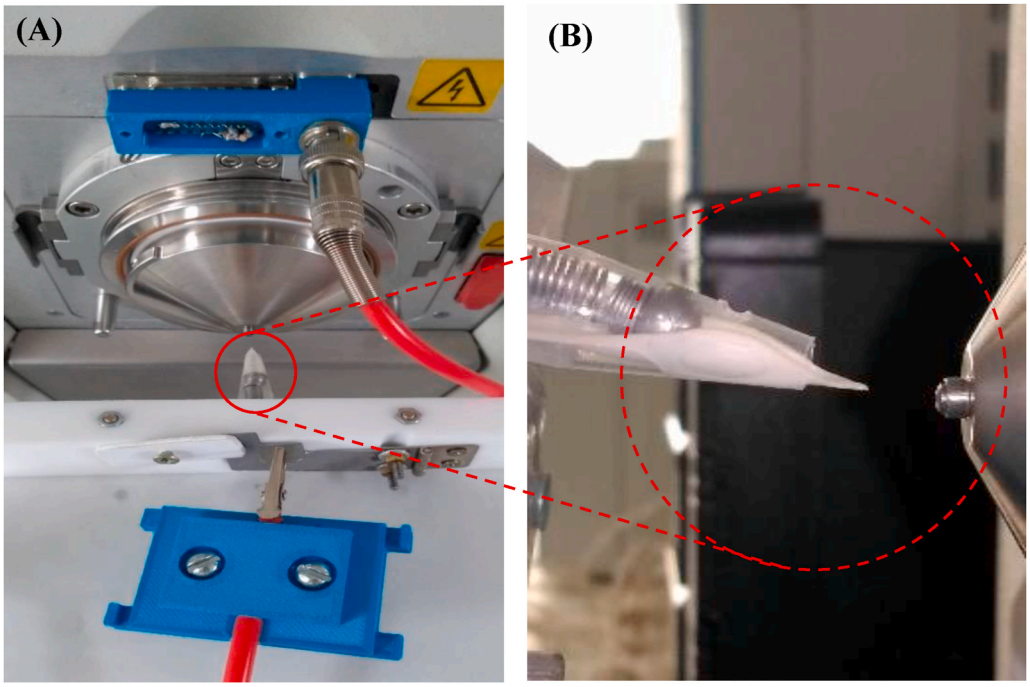

Fig. 2. Top (A) and side (B) views of paper spray ionization source and the developed supported paper configuration for MPEF-PS-MS.

intra- and interday precision and accuracy were assessed through six extractions of TCAs at three concentration levels (2, 6 and 10 ng mL^{-1}). For interday precision, two consecutive days were carried out. The extraction efficiency (EE) was estimated at these three concentration levels with six replicates for each according to Eq. (1). Hence, the method was applied to ten different spiked samples of human oral fluid at 6 ng mL^{-1} of each TCA with three replicates for each sample.

$$EE\ (\%) = \frac{peak\ area\ of\ the\ analyte\ extracted\ from\ oral\ fluid}{peak\ area\ of\ the\ analyte\ added\ to\ the\ oral\ fluid\ extract} \times 100 \quad \text{Eq. 1}$$

3. Results and discussion

Different multiphase electroextraction approaches have been described in recent years, such as the free solution presented by Raterink et al. [33] the conductive hydrogel proposed by Wuethrich et al. [49] the cellulose cone tip shown by Orlando et al. [40] and so on [50,51]. As mentioned in these works, the conductivity of the electrolyte solution, as well as the shape, length and porosity of the support used in the acceptor phase, greatly influences the electric resistance and therefore the extraction efficiency [40,41]. For this reason, a proof-of-concept study was carried out to certify that the chromatographic paper and its geometry (size and shape) were not limiting factors for the extraction performance. Crystal violet (a cationic dye) was chosen as the model compound for this test. The results (Fig. S2) show that CV effectively migrates to the chromatographic paper after 3 min of extraction. At the beginning of the extraction, after a few seconds, it was observed that the dye began coloring the tip of the paper where the electric charges were concentrated and was spread out over the time. Visually, the physical integrity of the chromatographic paper was maintained, confirming that it is a good support for multiphase electroextraction.

After the proof-of-concept test, the MPEF electroextraction procedure was optimized for the determination of five antidepressants by LC-MS/MS after a desorption step. The strategy was conducted in this way to validate the use of the chromatography paper with the shape presented in Fig. 1 before proceeding to the PS-MS analysis.

The results of the univariate studies were expressed as a mean of the extraction percentage for each analyte (n = 3) and are presented in Fig. 3. Initially, extractions with and without application of the electric field were performed. In Fig. 3 (A), it can be observed that the electric field was essential for improving (81 times in average) the TCA recovery when compared to the extraction without an electric field. Since the analytes were predominantly cationic in the donor phase (pH 6.50, the smallest pK_a of the TCAs is 9.20), they did not transfer extensively for the organic filter by diffusion and almost were not detected in the acceptor phase. This fact indicated that the transfer of the cationic form of the TCAs, from donor to acceptor phase, is mainly modulated by electromigration through the application of the electric field. Another observation was that beyond the higher extraction recovery obtained with the use of the electric field, a significant amount of analytes remained stuck in the organic filter (Fig. S3). This is in accordance with results that demonstrated that the partition equilibrium of the analyte (neutral and ionized forms) between the immiscible phases is displaced when ions migrate from the donor to acceptor phase [52].

The type, pH and concentration of the electrolyte in the acceptor phase must maintain adequate electrophoretic mobility (ionization) and ion exchange flux among the phases (conductivity and partition). The pH values of the electrolyte solutions used were sufficiently low and more than three units below the pK_a of the analytes [53]. Three different electrolyte solutions were tested for the acceptor phase and the best performance was observed for acetic acid (Fig. 3 (B)). This results are in accordance with previous studies of electromembrane extraction with free liquid membranes [54,55]. We could suppose that the partition (electrolyte solution in the acceptor phase-organic filter) of acetate or even formate ions is certainly greater than that observed for chloride ions. However, the amount of TCAs found in the organic filter (Fig. S4) was higher when HCl was used. One hypothesis for this observation is the change in the pH of the HCl electrolyte solution due to its low buffer capacity during the electrolysis process. Another possible explanation is the lack of electromigration of TCA-chloride in the organic filter due to the high stability of this neutral ion pair [52].

Regarding the electrolyte support of the acceptor phase, two types of chromatographic paper with the same composition but different thicknesses were compared. The 3 MM Chr is thicker than 1 Chr (0.34 *versus* 0.18 mm) and, consequently, the former can sustain a larger quantity of

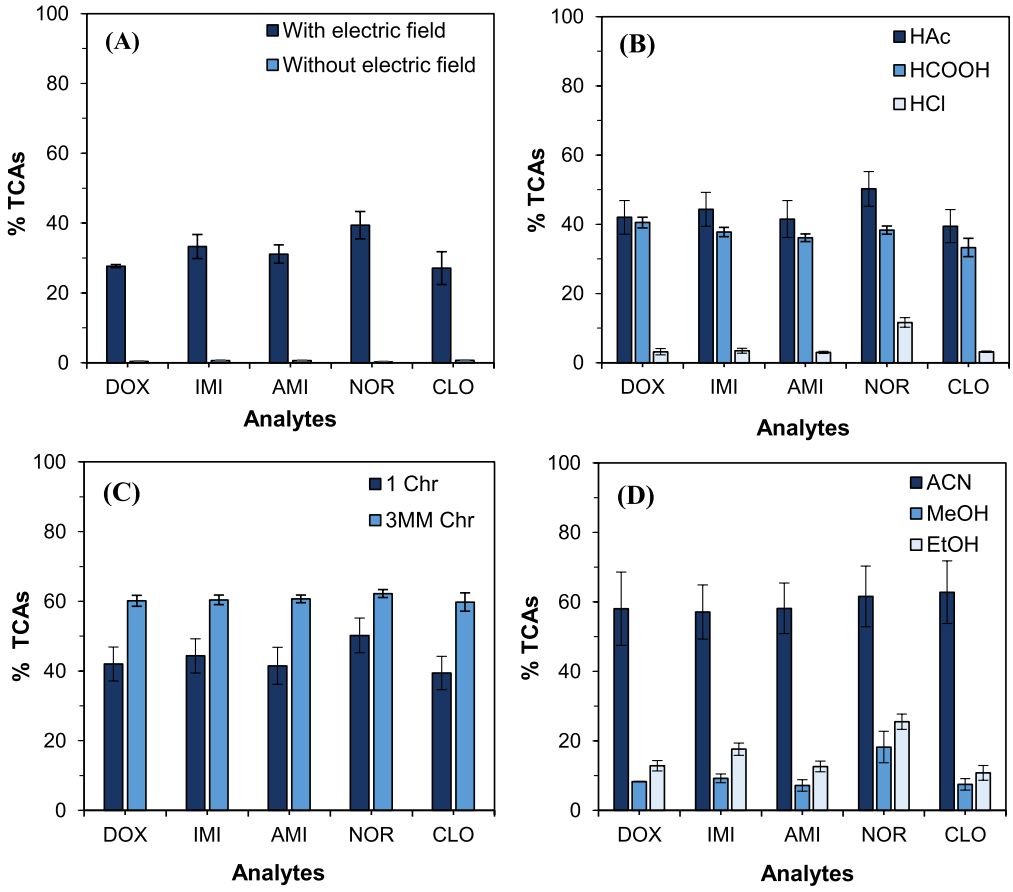

Fig. 3. Effect of electric field application (A), electrolyte solution type of the acceptor phase (B), paper thickness used as a sorbent in the acceptor phase (C) and polar organic solvent present in the donor phase (D) on TCA recoveries. General conditions: extraction voltage, 300 or 0 V; extraction time, 10 min; donor phase, 1 mL of aqueous solution spiked with TCAs at 0.5 μg mL^{-1} each; organic filter, 750 μL of 1-octanol; acceptor phase, chromatographic paper soaked with acid solution. HAc: acetic acid at 400 mmol L^{-1} pH 2.5; HCOOH: formic acid at 400 mmol L^{-1} pH 2; HCl: hydrochloric acid at 10 mmol L^{-1} pH 2; ACN: acetonitrile; MeOH: methanol; EtOH: ethanol. Error bars represent the standard deviation (n = 3).

electrolyte solution and maintain the electromigration for a longer period. This prediction was confirmed by the results presented in Fig. 3 (C), where the thicker chromatographic paper demonstrated an average improvement of 140% for all five TCAs.

For many MPEF extraction approaches described in the literature, polar organic solvents are added to the donor phase (sample) [56] to reduce the interfacial tension and improve the kinetics of the mass transfer of analytes between phases [33,40]. To increase the extraction of TCAs, acetonitrile, methanol and ethanol were evaluated. The best result was obtained with acetonitrile (Fig. 3 (D)). This observation can be attributed to the combination of the good partition between the aqueous and organic phases, as well the better capacity of solvation of the cationic TCAs by acetonitrile.

After the univariate studies, a Box-Behnken design was carried out to optimize the extraction time, electric potential and acetonitrile percentage added to the donor phase. These three are well known as some of the most significant parameters for improving the EE in MPEF extractions. Among them, the acetonitrile percentage presented the strongest influence in the process, followed by the extraction time and the electric potential. The response (analytical signal) was improved with the increase of all variables studied (Table S2). By comparing the response of the optimal conditions (300 V/18 min) versus the initial conditions (300 V/10 min), we conclude that, for our purpose, the desire to improve the analytical signal does not justify a much longer extraction time (Fig. S5). Therefore, it was decided that the initial conditions of 10 min of extraction time, 300 V of applied voltage and 50% ACN v/v in the donor phase would be maintained for evaluation of the performance of the MPEF-LC-MS/MS method. At these conditions, a maximum and average electric current of 0.72 and 0.48 mA, respectively, were observed.

To ensure that the chromatographic paper is suitable for the extractions, some figures of method were estimated, and the results are summarized in Table 2. The precision and accuracy were satisfactory according to the analytical guide. The LOD and LOQ values were comparable with other studies in the literature in which TCAs were determined in blood [57–59], urine [58–61] and saliva [59] through chromatography techniques and sample preparation assisted by an electric field (Table S3). No signal of interferents was observed in the chromatograms of the blank extract, demonstrating the selectivity of the method (Fig. S6).

The matrix effect was investigated by comparing the curves constructed in the desorption solvent and in the extracts of synthetic and human oral fluids, two by two, through the F- and t-tests at a confidence level of 95% and the statistical tests demonstrated that the matrix effect was not significant ($p > 0.05$) (Fig. S7). High selectivity and the absence of a matrix effect are important advantages of electroextraction techniques and have already been demonstrated in other works [41,56,62,63]. These characteristics are essential for direct analysis approaches, such as PS-MS, where the clean-up of the sample can reduce many problems regarding the signals of interferents or the accumulation of dirt on the mass spectrometry entrance and the frequent need of stops for maintenance.

The validation experiments were carried out in synthetic oral fluid; however, it is well known that the oral fluid may vary greatly according to the sex, age, diseases and so on. For this reason, the fortified samples of oral fluids from ten different volunteers were analyzed by the MPEF-LC-MS/MS method and it was observed that the TCA analytical signals were similar among samples and also to the analytical signal obtained with the synthetic oral fluid (Fig. S8), which reinforces the effective clean-up obtained by MPEF.

The first development to couple multiphase electroextraction to PS-MS analysis was the porous support. In 2019, Orlando described a

Table 2
Figures of merit for the MPEF-LC-MS/MS method.

Analyte	Linear regression equation (2–12 ng mL^{-1})	R^{2a}	LOD^{b}	LOQ^{b}	Accuracy (%)^{c} (RSD$_{intra\ assay}$; RSD$_{inter\ assay}$; %)			Extraction efficiency (%)^{d} (RSD; %)		
			(ng mL^{-1})		2 ng mL^{-1}	6 ng mL^{-1}	10 ng mL^{-1}	2 ng mL^{-1}	6 ng mL^{-1}	10 ng mL^{-1}
DOX	108.23 x + 85.40	0.9953	0.25	0.84	80 (12; 25)	96 (10; 11)	104 (9; 7)	51 (8)	51 (8)	53 (8)
IMI	341.64 x – 30.33	0.9962	0.13	0.44	81 (5; 8)	107 (9; 10)	108 (8; 7)	43 (4)	52 (8)	54 (7)
AMI	107.08 x + 7.69	0.9972	0.21	0.72	104 (13; 26)	103 (5; 13)	108 (11; 11)	54 (12)	59 (5)	57 (3)
NOR	94.60 x + 25.19	0.9928	0.17	0.57	75 (20; 18)	90 (5; 9)	100 (9; 8)	49 (16)	56 (5)	63 (10)
CLO	255.74 x + 107.64	0.9981	0.13	0.44	77 (5; 14)	99 (7; 10)	111 (13; 12)	42 (3)	47 (6)	52 (11)

[a]n = 18. [b]n = 10. [c,d]n = 6. DOX: doxepin; IMI: imipramine; AMI: amitriptyline; NOR: nortriptyline; CLO: clomipramine.

cellulose cone tip, such as that presented in Fig. 1 (C). This type of support was efficient for electroextraction; however, its low superficial area was not able to support an adequate amount of solvent to form a stable spray during PS-MS. The conventional triangle paper used in the PS-MS analysis is too short to contact the electrode and organic filter simultaneously. Thus, an adaptation to be suitable for both situations was necessary (Fig. 1 (C)). However, the longer shape of the paper developed for MPEF made handling difficult and PS-MS requires a significant manipulation for arranging it in front of mass spectrometry. Thus, the support approach described in Fig. 1 (A) was used to stabilize and reduce the variation in positioning. The support was made with a conventional polypropylene micropipette 5 mL tip. By cutting carefully the tip end with a sharp knife and making a little hole with a needle to drop the solvent used in PS-MS, the reusable support could be easily, and custom made.

The proof of concept and figures of merit for the MPEF-LC-MS/MS method demonstrate that the size and shape of the chromatographic paper is reliable to extract TCAs from oral fluid with reproducibility, selectivity and effectivity.

After these studies and using the same optimized conditions described for figures of merit estimation, the TCAs extracted from synthetic oral fluid were directly analyzed by the PS-MS approach. Fig. 4 (A) depicts the mass spectra with the intensity signals of the analytes and IS added to the sample. Using the TCA/IS ratio, RSD values lower than 19% for nortriptyline and lower than 14% for the others (n = 3) (Fig. 4 (B)) were observed. Variation in PS-MS analysis is common, and this problem is greatly reduced using isotope-labeled analytes. However, the use of just one analogue compound as the IS for all analytes and the few variations observed in the analytical signal after MPEF-PS-MS demonstrates that this approach could be applied in routine analysis without additional difficulty. In addition, adequate precision for a preconcentration of least 3.5-fold (based on a 35% recovery average, 500 µL of sample and 50 µL paper spray solvent volumes used) was observed. Although a relatively high concentration was evaluated (2 µg L^{-1}) for the TCAs, better sensitivity by PS-MS could be obtained. The sensitivity is strongly dependent to the mass spectrometry used and, in our case, the equipment employed was not state of the art. Undoubtedly, coupling the electroextraction with PS-MS (MPEF-PS-MS) brings advantages, such as the reduction of ion suppression, cost, maintenance and higher analytical frequency in relation to conventional PS-MS or LC-MS/MS.

4. Conclusions

The innovative chromatographic paper format and supports presented in this work were suitable for the effective extraction of tricyclic antidepressants from oral fluid, assisted by electric fields. The validation parameters of the MPEF-LC-MS/MS method demonstrated that extraction in this format can reduce the matrix effect and is suitably precise and accurate. The direct coupling of electroextraction to PS-MS demonstrated that there was no significant difficulty in interfacing both techniques, particularly when using the support made of micropipette tips. The intense and reproducible PS-MS signals reinforced that the approach presented here is promising for routine analysis, combining the best, well-established advantages of this sample preparation and analytical technique, such as the high analytical frequency, low cost of consumables, selectivity, and so on.

Author contributions

Millena Christie Ferreira Avelar, Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft. Clesia Cristina Nascentes, Supervision, Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft. Ricardo Mathias Orlando, Supervision, Conceptualization, Methodology, Investigation, Writing-Reviewing and Editing, Resources.

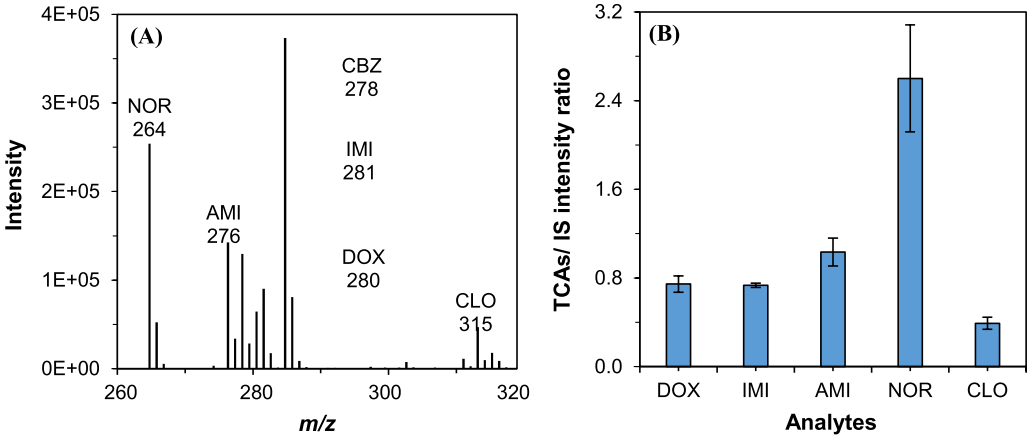

Fig. 4. Paper spray mass spectrometry analysis of TCAs after MPEF extraction. Mass spectrum of TCA mixture (A) and their intensities ratio with the internal standard (B). General MPEF conditions: applied voltage, 300 V; time, 10 min; donor phase, 1 mL of synthetic oral fluid spiked with TCAs and cyclobenzaprine (internal standard) all at 2 µg mL^{-1}; organic filter, 750 µL of 1-octanol; acceptor phase, chromatographic paper 3 MM Chr soaked with 70 µL of acetic acid at 400 mmol L^{-1}. General PS-MS conditions: ionization voltage, +4 kV; capillary temperature, 375 °C; paper distance from MS inlet, 5 mm; spray solvent, 50 µL of methanol: isopropanol 95.5: 4.5 with 0.1% formic acid (v/v/v). Error bars represent the standard deviation (n = 3).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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