

SU8/glass microchip capillary electrophoresis integrated with Pt electrodes for separation and simultaneous detection of phenylephrine and acetaminophen

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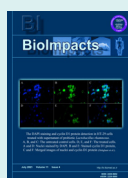
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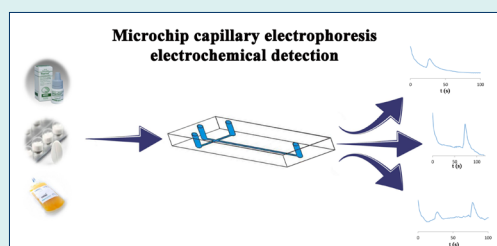
Abstract

Introduction: A new microfluidic-based method with electrochemical detection was developed for the simultaneous quantification of acetaminophen (AP) and phenylephrine (PHE) pharmaceuticals in the human blood and pharmaceuticals (e.g. tablet and drop).

Methods: The separation was achieved on a SU8/glass microchip with a 100 μm Pt working electrode that was positioned out of the channel and 2-(N-morpholino) ethanesulfonic acid was used as a running buffer (pH 7, 10 mM). Home designed modulated high voltage power supply and dual time switcher was used for controlling the injection and separation of the analytes in the unpinched injection mode.

Results: The injection was carried out using +750 V for 7 seconds, and the separation and detection voltages were set at +1000 V and +0.9 V, respectively. Critical parameters such as detection potential, buffer concentration, injection, and separation voltage were studied in terms of their effects on the resolution, peak height, and migration times. For each analyte, the correlation coefficients were over 0.99 (n=6). The developed microchip was able to detect AP and phenylephrine simultaneously with the limit of detection of 7.9 and 5.2 ($\mu\text{g/mL}$) respectively for PHE and AP and excellent linear range of 10-200 ($\mu\text{g/mL}$). The recovery of the drugs ranged from 96% to 103%, while the repeatability of the method through inter- and intra-day was lower than 7%.

Conclusion: The developed method offers several advantages, including easy sample pretreatment process, simplicity, very fast analysis compared to other typical chromatographic methods. Thus, the proposed microfluidic-based method is proposed to be used as a time- and cost-effective monitoring method for the analysis of AP and PHE.



Introduction

Acetaminophen (AP) (N-acetyl p-aminophenol), known as paracetamol, was introduced as an antipyretic/analgesic agent by Von Mering in 1893.¹ Since then, it has been used in the clinic as an analgesic and a major ingredient in numerous cold and flu medications. The effects of AP on neurons and its therapeutic potential in neurodegenerative diseases, particularly Alzheimer's disease have also been reported.² Although AP is a safe medicine under clinical doses, its overdose may cause fatal hepatotoxicity and

nephrotoxicity.

Phenylephrine hydrochloride (PHE) ((R)-1-(3-hydroxyphenyl)-2-methyl-aminoethanol hydrochloride) directly affects adrenergic receptors; it has been used in the treatment of hypertension, rhinitis, and sinusitis, in the symptomatic relief of cold symptoms. It is used in the formulations of eye-washers, nasal decongestants and syrups.³ The overdose of PHE may result in hypertension, cerebral hemorrhage, palpitation, and even sever/fatal cardiovascular reactions.



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PHE and AP are widely used in diseases accompanied by a cough, pain, and fever such as the common cold and other viral infections as an analgesic, antipyretic, decongestant, antihistamine, and antitussive.

Therefore, based on their clinical importance standpoint, the real-time detection of AP and PHE in biological fluids (e.g., blood, urine) has led to the development of a variety of determination approaches.⁴

Various methods have been reported for monitoring AP and PHE, including electrochemical,⁵⁻⁷ spectroscopic,^{8,9} chromatographic,¹⁰ and capillary electrophoresis methods.^{11,12} Both AP and PHE contain a phenolic hydroxyl group, which is electrochemically active and can be oxidized. Because of being simple and sensitive with good stability and low cost, the electrochemical techniques appear to be the method of choice for the detection of AP and PHE alone or in combination. In most bare solid electrodes, the oxidation peak potentials of AP and PHE show overlap. Thus, their simultaneous determination without separation or modification of the electrode surface is not plausible.¹³ For the simultaneous detection of AP and PHE, various HPLC methods have been reported^{14,15} and a few other analytical methods have also been stated for simultaneous determination of the mentioned analytes.¹⁶

In 1997, Manz group introduced a new though microfluidic-based analytical system, which is called micro total analytical systems (μ TAS).¹⁷ In the early stages of the development of μ TAS, glass or quartz was the main material for constructing microfluidic-based devices. Over time, various materials have been used for the construction of devices, including polymers like poly dimethyl siloxane by lithography method,¹⁸ and polymethyl methacrylate by hot embossing method.¹⁹ These devices were coupled with various detection systems such as MS-spectrometer,²⁰ UV-Vis,²¹ capacitively-coupled contactless conductivity detection (C4D),^{22,23} laser-induced fluorescence²⁴ and electrochemical methods.^{25,26} Of these, electrochemical systems have various advantages, including miniaturization, reproducibility, cost- and time-effectiveness, and compatibility and integration with microdevices.

In the present study, we developed an on-chip electrophoretic method with electrochemical detection for the separation and determination of AP and phenylephrine in pharmaceuticals and human blood plasma.

Materials and Methods

Chemicals and reagents

Analytical grade reagents and solvents were used for all the experiments carried out without more purification. AP, phenylephrine, and 2-(N-morpholino)ethanesulfonic acid (MES) were purchased from Sigma-Aldrich (St. Louis, USA). Sodium hydroxide, acetonitrile, and sodium perchlorate were purchased from Merck (Darmstadt,

Germany). Ultrapure water and human plasma were obtained respectively from Shahid Ghazi pharmaceutical Co. (Tabriz, Iran) and the Iranian Blood Transfusion Organization (Tabriz, Iran). About 1000 mg/L of different analytes were prepared in a 10 mL volumetric flask, stored at 4°C and in a dark place for no more than one month. An appropriate concentration of analytes was daily prepared by dissolving suitable amounts of analytes in the running buffer.

Running buffer (20 mM) was prepared by dissolving 2-(N-morpholino)ethanesulfonic acid in an appropriate volumetric flask, adjusting the pH by NaOH at 7. Other concentrations were prepared by diluting in deionized water and after adjusting pH, the running buffer passed through 0.45 μ m syringe filter (Membrane solutions, Kent, USA).

Apparatus and set-up design

The SU8-glass microchips were purchased from Micrux Technologies (Oviedo, Spain). These chips consist of cross-shaped channels with a 40 mm long separation channel (i.e., between the running buffer and detection reservoirs) and a 10 mm injection channel (i.e., between the sample and sample waste reservoirs) with 5 mm side-arms. SU-8 layer has respectively 20 and 50 μ m depth and width and is supported by a 2 mm glass substrate. Pt integrated electrodes with Ti substrate layer implanted in the detection reservoir and out channel approach were used for the determination of analytes. The width of the working, auxiliary and pseudo-reference electrodes was 100 μ m. A methacrylate holder (18 \times 13 \times 2 cm) was used for accommodating the chip. The chip was inserted in the holder and two rectangular pieces of the holder were fixed with four screws. A high voltage power supply with a modulated dual time switcher (Molian-Toos Co., Mashhad, Iran) was used to apply high voltages. Autolab PGSTAT 30 (Metrohm, Herisau, Switzerland) was used as a detector and data recorder. A pH meter (Metrohm model 744, Herisau, Switzerland) was used for the pH measurements. All the electrophoretic experiments were carried out in the Faraday cage in order to eliminate any environmental noises.

Pretreatment of the channels

Before performing the electrophoretic procedure, the channels should have been rinsed and activated. SU8 possesses a hydrophobic surface. Thus, first, NaOH (0.1 M) was run through the channels for hydrophilization and activation of the channel surfaces. In fact, the surfaces are charged negatively, and electroosmotic flow (EOF) can appear after treating with NaOH. Next, the channels were rinsed with deionized water to wash the NaOH out of the channel. Finally, the channels were rinsed with running buffer in order to carry out the electrophoresis procedure. These steps were carried out at the beginning of the day

for 15, 10, and 15 minutes, respectively by a water jet vacuum generator.

Electrophoretic procedure

A high-voltage power supply, with an adjustable voltage range between 0 and +2000 V, was used for the electrophoretic separations. The unpinched method was used for the injection and separation of the analytes. In a brief, for the injection, the high voltage was applied to the sample reservoir while the sample waste reservoir was at the ground state for a definite time. During this period, analytes were electro-moved to the sample waste reservoir to fill the cross-section of the channels. At the separation step, the high voltage was applied between the buffer and detection reservoirs. As a result, the analytes were moved to the end of the channel and separated proportionally to their m/z (m and z denote for the mass and the charge of the analytes, respectively). Other reservoirs were at the floating state when no voltage was applied.

Real sample analyses

Sample preparation

For the human serum analysis, a designated amount of analyte was spiked to 1.0 mL of the serum sample and vortexed for 30 seconds. Then, 2.0 mL of ACN was added to deproteinize samples. The mixture was centrifuged at 10000 rpm for 25 minutes. Then, the supernatant was filtered through a 0.45 μm syringe filter and transferred into another microtube and dried with an N_2 stream. The residue was dissolved in 100 μL of running buffer.

For the pharmaceutical tablet sample preparation, the contents of 5 tablets were accurately powdered in a mortar and a weight equivalent to one tablet was dissolved in 50 mL of deionized water. Then, the solution was vortexed for 30 minutes and allowed to stand for 15 minutes. Afterward, the supernatant was centrifuged for 10 minutes at 10000 rpm. Finally, the solution was filtered through a 0.45 μm syringe filter to eliminate the particle. About 25 μL of the prepared solution was transferred to a 5 mL flask and diluted with the running buffer (pH 7, 10 mM).

For the preparation of pharmaceutical drops, nasal drop (100 μL) was diluted in the running buffer up to 5 mL (MES, pH 7, 10 mM).

HPLC analyzing conditions

Chromatographic analysis was performed using a Knauer unit equipped with a diode-array detector (DAD) and a 20 μL injection loop. EZChrom elite ver. 3.1.7 software was used to control the process of experiments. Nucleodur C18 reversed-phase column (250 mm \times 4.6 mm length, 5 μm particle size) was used at 35°C as the stationary phase. Gradient elution was initiated with acetonitrile-sodium perchlorate solution (pH 3, 0.01M; 5:95 v/v%), and within 8 minutes gone to 60% acetonitrile with the flow rate of 1.4 mLmin⁻¹. The absorption wavelength was set at 204 nm.

Microchip capillary electrophoresis (MCE) analyzing conditions

MES-NaOH buffer (10mM, pH 7) was used as the running buffer and the unpinched injection method was used for the injection of the analytes to the channel. For the injection, +750 V was applied to the sample reservoir for 5 seconds while the sample waste reservoir was at the ground state. Then, +1000 V was applied between the buffer and the detection reservoirs for the separation. The optimum detection potential was equal to the +0.9 V vs. Pt pseudo-reference electrode.

Safety consideration

To avoid any electrical shock, the high-voltage power supply and related connectors should be handled with extreme care.

Results and Discussion

Amperometric detection

Through the migration of the analytes to the end of the channel, they separate from each other based on their m/z rates and collision to the surface of the working electrode. By the collision of the analytes, they lose an electron and turn to an oxidized state. The analytes were injected into the channel and the effect of oxidation potential was studied. As shown in Fig. 1, at +0.9 V, the hydrodynamic curves reach a plateau. As a result, +0.9 V vs. Pt pseudo-reference electrode was chosen as the optimum potential for the detection.

Effect of buffer concentration

The effect of the running buffer concentrations on the response and separation of PHE and AP was studied based on the background electrolytes containing 5, 10, 15 and 20 mM MES buffer (pH=7). The results, peak current (I_p) and migration time (t_m) as the function of the buffer concentration, are shown in Fig. S1A and B (Supplementary file 1), respectively. As shown in Fig. S1A (Supplementary file 1), with the increase of the buffer

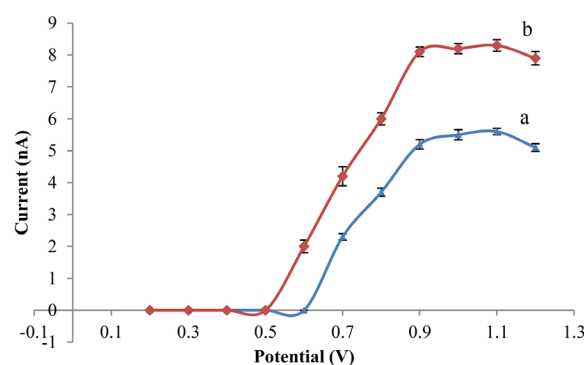


Fig. 1. Hydrodynamic voltammograms for PHE (a), AP (b) obtained with a Pt working electrode. Other conditions: Concentrations= 50 $\mu\text{g/mL}$, V_{inj} = +650 V, T_{inj} = 5, V_{sep} = +900 V, running buffer: 20 mM MES (pH=7).

concentration, the peak height is decreased, in large part because of increasing of the ionic strength. An increase in MES concentration resulted in an increase in t_m for both analytes (Fig. S1B, Supplementary file 1), which is speculated to be a consequence of the decreasing of the EOF order.

Effect of injection voltage and time

The effect of injection time (T_{inj}) on the current and shape of the peak was examined by varying the injection time (T_{inj}) from 1 to 8 seconds. As shown in Fig. S2 (Supplementary file 1), no peaks were observed in the range of 1 to 3 seconds for PHE. This means that it takes at least 5 and 7 seconds respectively for AP and PHE to fill the cross-section of the channels and after this period, increasing the time had no effects on the peak current.

In order to investigate the injection voltage, various potentials (+650, +750, +850 and +950 V) were applied for injecting the analytes into the cross-section. In fact, with the increasing of the voltage, analytes appeared to move more rapidly to the sample waste reservoir through the cross-section, and after filling the cross-section and stabilizing of the analytes' concentrations, the application of more voltages was not necessary. Therefore, 7 seconds and +750 V were chosen as the optimum conditions for the injection time (T_{inj}) and voltage, respectively.

Effect of separation voltage

The effect of the separation voltage was examined when the separation voltage increased; the migration time of PHE and AP decreased and the peak shapes became sharper. As shown in Fig. 2, by increasing the separation voltage, the baseline current increased (i.e., 2 ± 0.04 nA at +800 V / 7 ± 0.2 nA at +1200 V). On the basis of separation speed, efficiency, and stability, +1000 V was chosen as the optimum separation voltage.

Calculation of theoretical plates and relative separation

Having used the optimum detection conditions,

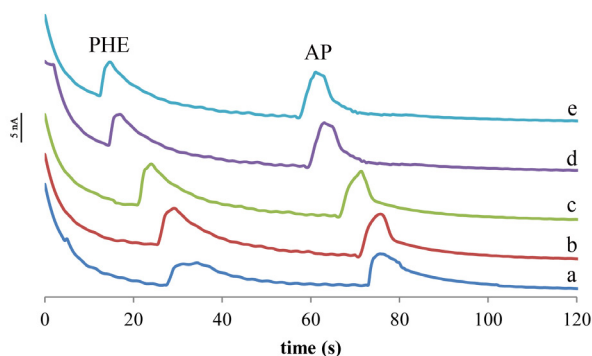


Fig. 2. Effect of separation voltage on the analytes; (a) +800, (b) +900, (c) +1000, (d) +1100, and (e) +1200. Other conditions: Concentrations= 50 $\mu\text{g}/\text{mL}$, V_{inj} = +750 V, E_d = +0.9 V v.s. Pt pseudo-reference electrode, running buffer: 10 mM MES (pH=7).

theoretical plates number and resolution were calculated according to equations 1 and 2, respectively.

$$N = 5.54 \times (t_m / w_{1/2})^2 \quad \text{Eq. (1)}$$

Where, $w_{1/2}$ and t_m are the half-peak width and migration time, respectively. The N values for PHE and AP were 24831 and 37129 N/m, respectively.

$$R_s = 2 \times (t_{m2} - t_{m1}) / (w_1 + w_2) \quad \text{Eq. (2)}$$

Where, w and t_m are the peak width and migration time, respectively. The R_s value was equal to 3.7 for PHE/AP pairs.

Repeatability (inter-day and intra-day assessments)

The intra- and inter-day repeatability of the method was investigated through the replicate ($n = 5$) analyses of standard samples spiked in two levels of PHE and AP. The relative standard deviation (RSD %) of the peak areas for both analytes under optimum conditions was calculated (Table 1). It is worth to mention that the obtained values of RSD are low enough to guarantee the precision of the proposed methodology.

Linearity

The calibration curves were established using six different concentrations in the range of 20-100 $\mu\text{g}/\text{mL}$ for PHE and 10-100 $\mu\text{g}/\text{mL}$ for AP. The electrochemical response of the detector was plotted as a function of the concentrations of the analytes under the optimum conditions (i.e., 10 mM MES-NaOH, pH=7, as background electrolyte, and +1000 V, +750 V and +0.9 V as the injection, separation and detection potential, respectively). The results are shown in Fig. 3, by increasing the concentration of analytes, the peak areas increases and linear relationships between the concentration and peak areas can be obtained. Analytical figures of merit (i.e., migration time, linear range, slope, correlation coefficient and limits of detection) for each analyte are summarized in Table 2.

In comparison to other methods reported previously, based on our findings, this microfluidic-based method possesses its unique advantages. To the best of our best knowledge, in most of the published works, AP has been analyzed with its metabolite (e.g. p-aminophenol) or with some other antipyretic/analgesic compounds.²⁷⁻²⁹

Table 1. Precision data: intraday results of five injections of the PHE and AP at concentrations of 50 and 100 μM and interday results of five mixture injections at 50 μM level

RSD %	PHE	AP
Intraday, 50 $\mu\text{g}/\text{mL}^{-1}$ level	6.35	4.1
Intraday, 100 $\mu\text{g}/\text{mL}^{-1}$ level	3.22	3.42
Interday	2.73	2.14

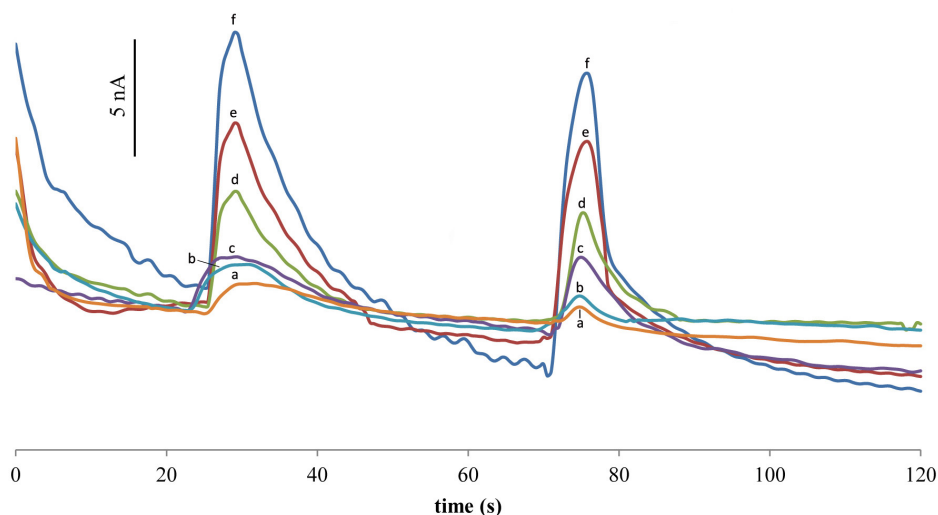


Fig. 3. Calibration curves for PHE; (a)20, (b)30, (c)40, (d)50, (e)70, and (f)100, and AP (a)10, (b)30, (c)40, (d)50, (e)70, and (f)100 $\mu\text{g/mL}$. Other conditions: $T_{\text{inj}}=7\text{s}$, $V_{\text{inj}}=+750\text{ V}$, $E_d=+0.9\text{ V}$ vs. Pt pseudo-reference electrode, running buffer: 10 mM MES (pH=7).

Further, PHE and AP are very similar compounds and their determination without separation seems to be a very challenging issue. In most analyses, instruments such as HPLC have been used for the separation. In fact, in such approaches, simple and cost-effective techniques such as electrochemical detection approaches have rarely been exploited for the simultaneous detection of the mentioned compounds. Other approaches like spectroscopic devices need chemometric methods for the separation of PHE and AP.

Application

The engineered set-up was used for the separation and determination of PHE and AP in the pharmaceutical tablets, nasal drop and human blood plasma in comparison with HPLC-DAD method.⁴ Fig. 4 illustrates the electropherograms and chromatograms of the results for analyzing the dosage forms such as tablets and drop. The recovery experiments were performed on phenylephrine nasal drop (0.25 w/v%) and AP tablet (500 mg/tablet) to evaluate matrix effects. The standard-solution additions yielded excellent recovery values (97.2–103.7% and 96.2–101.3% for PHE and AP, respectively). These results indicate that there were no important matrix interferences for the samples analyzed by the proposed microfluidic-based method. The presented data in Table 3 reveal that the obtained results by this proposed method are in consensus with the required values. Based on

the statistical analyses, the resulting t test values at the confidence level of 95% are much smaller than the critical one (4.30, $\alpha = 0.05$), indicating that PHE and AP contents detected by the present method, is in good agreement with the claimed value.

The PHE and AP peaks were not observed in the diluted serum sample, implying that the amount of PHE and AP is lower than our detection range. Therefore, known concentrations of analytes were spiked into the serum sample at two levels and analyzed by proposed and HPLC-DAD methods.

Table 2. Analytical parameters corresponding to the calibration curves for the PHE and AP

	t_m (s)	Sensitivity ($\text{pA}\times\text{s}/\mu\text{g/mL}$)	Linear range ($\mu\text{g/mL}$)	R^2	LOD ($\mu\text{g/mL}$)
PHE	30.4 \pm 0.4	0.9	20-100	0.994	7.9
AP	79.5 \pm 0.7	1.4	10-200	0.998	5.2

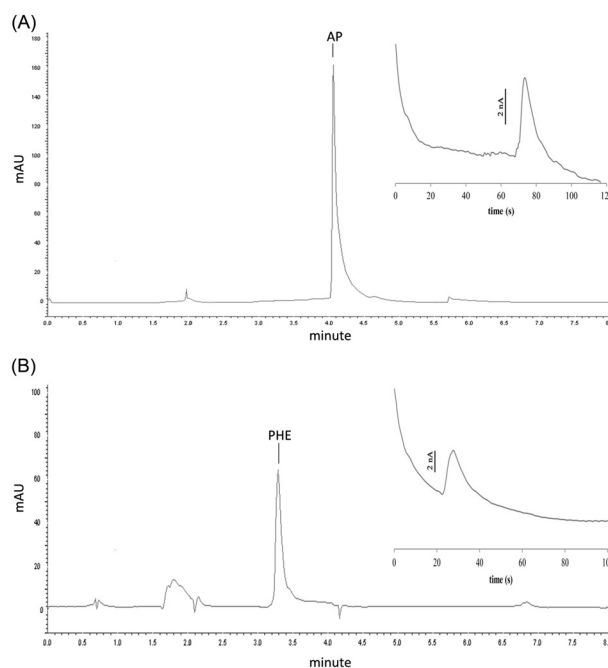


Fig. 4. Analysis of analytes by MCE-EC and HPLC-DAD. A) AP tablets by HPLC-DAD, Inset: AP tablets by MCE-EC. B) PHE nasal drop by HPLC-DAD, Inset: PHE nasal drop by MCE-EC.

Table 3. Results for determination of phenylephrine in the nasal drop and acetaminophen in oral tablet samples (n=5)

Sample	Label Claimed	Found by HPLC-DAD	Found by MCE-EC	Error % ^a
PHE nasal drop (Sina Darou, Iran)	25 mg/100 mL	24.97	24.94	-0.12
AP tablet (Chemi Darou, Iran)	500 mg/tablet	501.22	502.15	0.18

PHE: phenylepherin, AP: acetaminophen.

^a Average error (%) = $100 \times (\text{proposed method value} - \text{HPLC value}) / \text{HPLC value}$

Table 4. Results for determination of phenylephrine and acetaminophen spiked in human blood plasma

Concentration ($\mu\text{g/mL}$)	Found by HPLC-DAD		Found by MCE-EC		t-value ^a	
	PHE	AP	PHE	AP		
4	3.84	4.15	4.08	4.21	2.08	2.47
6	6.05	5.96	5.91	5.95	1.74	0.98

Note. ^a The confidence level is 95%.

Our findings (Table 4 and Fig. 5) approved that the proposed microchip capillary electrophoresis with electrochemical detection can simply be applied for the concurrent determination of PHE and AP in complex and real samples.

Conclusion

To the best of our knowledge, the proposed microchip capillary electrophoresis-electrochemical detection approach is the first prototype for the separation and simultaneous determination of phenylephrine and AP. Under optimum conditions, good linearity can be obtained for different calibration concentrations, making the analysis plausible in pharmaceuticals and human

blood serum. Taken all, the present microfluidic-based microchip device seems to be a simple, robust, and cost-effective detection approach for the simultaneous detection of analytes with structural similarities in comparison with the currently used methods such as HPLC-DAD technique.

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Funding sources

There is none to be clarified.

Ethical statement

There is none to be clarified.

Competing interests

None to be declared.

Authors' contribution

AA, YO, and MM developed the idea. AA acted in developing the analytical method, data analysis, drafting the manuscript. MM and

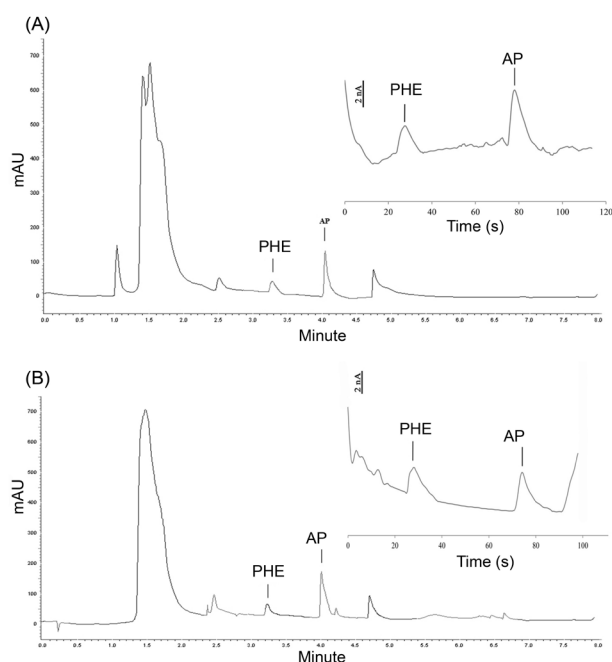


Fig. 5. Results for the determination of PHE and AP in the plasma at two concentration levels. A) analyzed by HPLC-DAD (4 ppm), Inset: analyzed by MCE-EC (4 ppm). B) analyzed by HPLC-DAD (6 ppm), Inset: analyzed by MCE-EC (6 ppm).

Research Highlights

What is the current knowledge?

✓ Lab-on-a-chip devices based on electro-moving and electrochemical detection systems were studied for chemical analyzing.

What is new here?

✓ The proposed method has the advantage of needing a very simple sample pretreatment and being faster than a typical HPLC chromatographic method.

✓ The microchip showed excellent recovery repeatability with less than 7 % RSD inter- and intra-day.

✓ The limits of detection for the developed microchip were 7.9 and 5.2 ($\mu\text{g/mL}$) respectively for phenylephrine and AP and excellent linear range of 10-200 ($\mu\text{g/mL}$).

YO supervised the thesis project. HV acted as the advisor of the thesis project.

References

- Prescott LF. Paracetamol: past, present, and future. *Am J Ther* **2000**; 7: 143-7. <https://doi.org/10.1097/00045391-200007020-00011>
- Locke CJ, Fox SA, Caldwell GA, Caldwell KA. Acetaminophen attenuates dopamine neuron degeneration in animal models of Parkinson's disease. *Neurosci Lett* **2008**; 439: 129-33. <https://doi.org/10.1016/j.neulet.2008.05.003>
- Shama S. Spectrophotometric determination of phenylephrine HCl and orphenadrine citrate in pure and in dosage forms. *J Pharm Biomed Anal* **2002**; 30: 1385-92. [https://doi.org/10.1016/S0731-7085\(02\)00437-5](https://doi.org/10.1016/S0731-7085(02)00437-5)
- Palabıyık I, Onur F. The simultaneous determination of phenylephrine hydrochloride, paracetamol, chlorpheniramine maleate and dextromethorphan hydrobromide in pharmaceutical preparations. *Chromatographia* **2007**; 66: 93-6. <https://doi.org/10.1365/s10337-007-0324-5>
- Rezk MR, Fayed AS, Marzouk HM, Abbas SS. Potentiometric ion-selective electrodes for determination of cyclopentolate hydrochloride and phenylephrine hydrochloride in their challenging ophthalmic formulation. *J Solid State Electrochem* **2018**; 1-11. <https://doi.org/10.1007/s10008-018-4045-5>
- Beitollahi H, Movlaee K, Ganjali MR, Norouzi P, Hosseinzadeh R. Application of a nanostructured sensor based on graphene- and ethyl 2-(4-ferrocenyl [1, 2, 3] triazol-1-yl) acetate-modified carbon paste electrode for determination of methyl dopa in the presence of phenylephrine and guaifenesin. *Appl Organomet Chem* **2018**; 32: e4243. <https://doi.org/10.1002/aoc.4243>
- Ruiyi L, Haiyan Z, Zaijun L, Junkang L. Electrochemical determination of acetaminophen using a glassy carbon electrode modified with a hybrid material consisting of graphene aerogel and octadecylamine-functionalized carbon quantum dots. *Microchimica Acta* **2018**; 185: 145. <https://doi.org/10.1007/s00604-018-2688-7>
- Ragab MA, El Yazbi FA, Hassan EM, Khamis EF, Hamdy MM. Spectrophotometric analysis of two eye preparations, vial and drops, containing ketorolac tromethamine and phenylephrine hydrochloride binary mixture and their ternary mixture with chlorpheniramine maleate. *Bull Fac Pharm Cairo Univ* **2018**; 56: 91-100. <https://doi.org/10.1016/j.bfopcu.2018.03.004>
- Al-Shwaiyat MKE. Spectrophotometric Determination of Paracetamol by Reduction of 18-Molybdo-2-Phosphate Heteropoly Anion. *Jordan J Chem* **2013**; 8: 79-89.
- Li P, Xiao J, Liu J, Liu R, Bi K, Li Q. Development and full validation of a liquid chromatography-tandem mass spectrometry method for determination of carbinoxamine in beagle plasma and its application to a pharmacokinetic study. *J Chromatogr B* **2018**; 1093: 183-9. <https://doi.org/10.1016/j.jchromb.2018.05.002>
- Suntornsuk L. Recent advances of capillary electrophoresis in pharmaceutical analysis. *Anal Bioanal Chem* **2010**; 398: 29-52. <https://doi.org/10.1007/s00216-010-3741-5>
- Gomez MaR, Olsina RA, Martinez LD, Silva MaF. Simultaneous determination of dextromethorphan, diphenhydramine and phenylephrine in expectorant and decongestant syrups by capillary electrophoresis. *J Pharm Biomed Anal* **2002**; 30: 791-9. [https://doi.org/10.1016/S0731-7085\(02\)00362-X](https://doi.org/10.1016/S0731-7085(02)00362-X)
- Majidi MR, Pournaghi-Azar MH, Baj RFB. Graphene nanoplatelets like structures formed on ionic liquid modified carbon-ceramic electrode: As a sensing platform for simultaneous determination of dopamine and acetaminophen. *J Mol Liq* **2016**; 220: 778-87. <https://doi.org/10.1016/j.molliq.2016.05.034>
- Heydari R. A new HPLC method for the simultaneous determination of acetaminophen, phenylephrine, dextromethorphan and chlorpheniramine in pharmaceutical formulations. *Anal Lett* **2008**; 41: 965-76. <https://doi.org/10.1080/00032710801978137>
- Şenyuva H, Özden T. Simultaneous high-performance liquid chromatographic determination of paracetamol, phenylephrine HCl, and chlorpheniramine maleate in pharmaceutical dosage forms. *J Chromatogr Sci* **2002**; 40: 97-100. <https://doi.org/10.1093/chromsci/40.2.97>
- Kalambate PK, Srivastava AK. Simultaneous voltammetric determination of paracetamol, cetirizine and phenylephrine using a multiwalled carbon nanotube-platinum nanoparticles nanocomposite modified carbon paste electrode. *Sensors and Actuators B: Chemical* **2016**; 233: 237-48. <https://doi.org/10.1016/j.snb.2016.04.063>
- Manz A, Graber N, Widmer H. Miniaturized total chemical analysis systems: a novel concept for chemical sensing. *Sensors and Actuators B: Chemical* **1990**; 1: 244-8. [https://doi.org/10.1016/0925-4005\(90\)80209-1](https://doi.org/10.1016/0925-4005(90)80209-1)
- Duffy DC, McDonald JC, Schueller OJ, Whitesides GM. Rapid prototyping of microfluidic systems in poly (dimethylsiloxane). *Anal Chem* **1998**; 70: 4974-84. <https://doi.org/10.1021/ac980656z>
- Lee G-B, Chen S-H, Huang G-R, Sung W-C, Lin Y-H. Microfabricated plastic chips by hot embossing methods and their applications for DNA separation and detection. *Sensors and Actuators B: Chemical* **2001**; 75: 142-8. [https://doi.org/10.1016/S0925-4005\(00\)00745-0](https://doi.org/10.1016/S0925-4005(00)00745-0)
- Dahlin AP, Wetterhall M, Liljegren G, Bergström SK, Andrén P, Nyholm L, et al. Capillary electrophoresis coupled to mass spectrometry from a polymer modified poly (dimethylsiloxane) microchip with an integrated graphite electrospray tip. *Analyst* **2005**; 130: 193-9. <https://doi.org/10.1039/B414592E>
- Petersen NJ, Mogensen KB, Kutter JP. Performance of an in-plane detection cell with integrated waveguides for UV/Vis absorbance measurements on microfluidic separation devices. *Electrophoresis* **2002**; 23: 3528-36. [https://doi.org/10.1002/1522-2683\(200210\)23:20<3528::AID-ELPS3528>3.0.CO;2-5](https://doi.org/10.1002/1522-2683(200210)23:20<3528::AID-ELPS3528>3.0.CO;2-5)
- Brito-Neto JGA, Fracassi da Silva JA, Blanes L, do Lago CL. Understanding capacitively coupled contactless conductivity detection in capillary and microchip electrophoresis. Part 2. Peak shape, stray capacitance, noise, and actual electronics. *Electroanalysis* **2005**; 17: 1207-14.
- Brito-Neto JGA, Fracassi da Silva JA, Blanes L, do Lago CL. Understanding capacitively coupled contactless conductivity detection in capillary and microchip electrophoresis. Part 1. Fundamentals. *Electroanalysis: An International Journal Devoted to Fundamental and Practical Aspects of Electroanalysis* **2005**; 17: 1198-206. <https://doi.org/10.1002/elan.200503237>
- Schulze P, Ludwig M, Kohler F, Belder D. Deep UV laser-induced fluorescence detection of unlabeled drugs and proteins in microchip electrophoresis. *Anal Chem* **2005**; 77: 1325-9. <https://doi.org/10.1021/ac048596m>
- Vandaveer IV WR, Pasas-Farmer SA, Fischer DJ, Frankenfeld CN, Lunte SM. Recent developments in electrochemical detection for microchip capillary electrophoresis. *Electrophoresis* **2004**; 25: 3528-49. <https://doi.org/10.1002/elps.200406115>
- Asadpour-Zeynali K, Ahadzadeh I, Aghdam AA, Veladi H. A Simple and Cheap Method for Microchip Capillary Electrophoresis Construction. *Sensor Letters* **2016**; 14: 938-42. <https://doi.org/10.1166/sl.2016.3715>
- Chu Q, Jiang L, Tian X, Ye J. Rapid determination of acetaminophen and p-aminophenol in pharmaceutical formulations using miniaturized capillary electrophoresis with amperometric detection. *Anal Chim Acta* **2008**; 606: 246-51.
- He F, Liu A, Xia X. Poly (dimethylsiloxane) microchip capillary electrophoresis with electrochemical detection for rapid measurement of acetaminophen and its hydrolytate. *Anal Bioanal Chem* **2004**; 379: 1062-7.
- Wang J, Chatrathi MP, Tian B, Polsky R. Microfabricated electrophoresis chips for simultaneous bioassays of glucose, uric acid, ascorbic acid, and acetaminophen. *Anal Chem* **2000**; 72: 2514-8.