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A simple and sensitive CE method for the simultaneous determination of catecholamines in urine with in-column optical fiber light-emitting diode-induced fluorescence detection

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ABSTRACT

A simple and sensitive method has been developed for simultaneous analysis of three catecholamines: dopamine (DA), epinephrine (EP) and norepinephrine (NE) in urine by capillary electrophoresis (CE) coupled with in-column fiber-optic light-emitting diode-induced fluorescence detection (ICFO-LED-IFD). Fluorescein isothiocyanate was used as the fluorescence tagged reagent for derivatization of DA, EP and NE. The CE conditions for separation of these catecholamines were systematically investigated. It was found that catecholamines could be more effectively separated by adding β-cyclodextin (β-CD) and acetonitrile (ACN) to a background electrolyte (BGE) of sodium borate. The migration times are 10.61, 10.83 and 11.14 min for DA, EP and NE, respectively and the catecholamines are completely separated within 11.5 min under the optimal condition of a BGE containing 10% v/v ACN, 20 mM β-CD and 20 mM sodium borate (pH 9.5), and an applied voltage of 13 kV. The relative standard deviations of migration time and peak area for these catecholamines are less than 0.16 and 2.0%, respectively. The limit of quantifications (LOQs) for DA, EP and NE are 3.5, 1.0 and 3.1 nM whereas the limit of detections (LODs) for DA, EP and NE are 1.0, 0.3 and 0.9 nM, respectively. Our proposed CE method provides low LOQ and LOD values. This CE-ICFO-LED-IFD methodology has been successfully applied to analyze catecholamines in human urine samples with good accuracy and satisfactory recovery.

1. Introduction

Catecholamines are hormones produced by the adrenal glands located on kidneys. They are released into the blood during times of physical or emotional stress. The major catecholamines are dopamine (DA), epinephrine (EP) and norepinephrine (NE) composed of dihydroxyphenyl and amine moieties. They play an important role as neurotransmitters in the various physical activities of the cardiovascular, nervous and endocrine systems. They can also affect the body's metabolism and their content in blood and urine is closely related to high blood pressure, paraganglioma, pheochromocytoma, and other diseases [1]. As such, it is of particular importance to determine the body fluid content of catecholamines in clinical diagnosis.

For these reasons, different analytical methods have been reported for determination of catecholamines. So far, high-performance liquid chromatography (HPLC) is the most popular technique [2,3]. For instances, the analysis of catecholamines in human urine [4], rat adrenal gland [5] and bovine chromaffin cell cultures [6] by HPLC have been conducted. However, capillary electrophoresis (CE) is becoming an attractive alternative to determine catecholamines owing to its attributes of high separation efficiency, low sample consumption and relatively short analysis time. Several detection techniques including ultraviolet absorption [7–9], electrochemical [10–12], chemiluminescence [13–15], laser-induced fluorescence (LIF) [16,17], and mass spectrometry [18,19] coupled with CE have been explored to analyze catecholamines. Among these, LIF detection is the most sensitive technique for catecholamines but the cost of a laser is relatively high. Compared to laser, light-emitting diodes (LEDs) have their own unique advantages such as small size, low cost, long lifetime, stable output with less energy consumption, broad range of emis-

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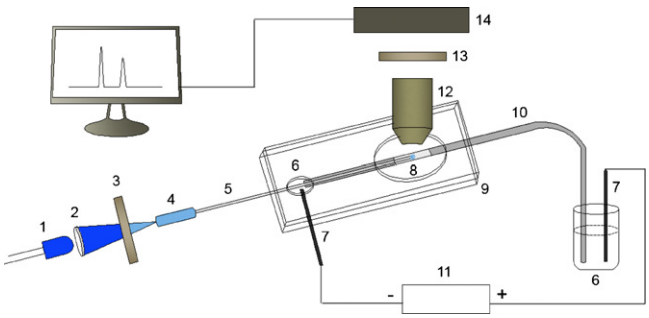

Fig. 1. Schematic of the CE-ICFO-LED-IFD system. (1) LED, (2) focusing lens (diameter: 6 mm; focus: 10 mm), (3) bandpass filter 1, (4) GRIN lens (diameter 3.0 mm), (5) optical fiber, (6) buffer reservoirs, (7) Pt electrodes, (8) detection window, (9) organic glass chip, (10) separation capillary, (11) high-voltage power supply, (12) 60× microscope objective, (13) bandpass filter 2, and (14) PMT.

sion wavelengths (280–1300 nm) and ease of operation. Thus, there is an increasing interest in exploring their uses and applications in CE [20].

In our past research effort, we have successfully developed a more cost-effective and sensitive detection system for CE, named as in-column fiber-optic light-emitting diode-induced fluorescence detection (ICFO-LED-IFD) [21]. In essence, this detection system involves coupling the light energy from a LED light source to the optical fiber which is inserted directly into the tail end of the separation capillary such that the excitation light is directly transmitted to the detection window. In this design, the light reflectance and scattering on the capillary surface is reduced thus lowering the background noise. On the other hand, LED usually produces a very broad emission band which can enter the photon detector to produce high background noise even though a cutoff filter is situated in front of the photomultiplier tube (PMT). It is often that many fluorophores possess a small Stokes' shift, and the LED's spectral emission can easily overlap with the fluorescence emission band; thus, the longer emission band of the LED can still reach the PMT together with the fluorescence signal. In this ICFO-LED-IFD design, only one cutoff filter situated in front of the PMT was used [21]. To a certain extent, the background noise still persists. In order to circumvent this drawback, two bandpass filters are proposed in this work: one bandpass filter is positioned behind the LED to select a narrower excitation light band; another bandpass filter is placed in front of the PMT to remove spectral interferences. As such, the detection system is improved to achieve lower background level and higher sensitivity of detection.

The main objective of this work is to develop a simple, sensitive and cost-effective ICFO-LED-IFD detection system for simultaneous determination of three catecholamines labeled with fluorescein isothiocyanate (FITC). The experimental conditions for analysis of the catecholamines have been systematically studied. Finally, the optimal CE condition was sought and the proposed CE-ICFO-LED-IFD methodology has been successfully applied to determine catecholamines in human urine sample with satisfactory results.

2. Experimental

2.1. Chemicals and reagents

Dopamine, epinephrine, fluorescein isothiocyanate, and norepinephrine were purchased from Sigma (St. Louis, MO, USA). 1-Heptanesulfonic acid sodium salt was obtained from Shandong Yuwang Industrial Co., Ltd. (Yucheng, Shandong, China). β-Cyclodextrin hydrate (β-CD), HPLC-grade acetonitrile (ACN) and methanol, and sodium tetraborate decahydrate ($Na_2B_4O_7{\cdot}10H_2O$) were from Kermel (Tianjin, China). Purified water from a Milli-Q-RO4 water purification system (Millipore, Bedford, MA, USA) with a resistivity higher than 18 MΩ cm was used to prepare all aqueous solutions. All other chemicals and organic solvents of analytical-reagent grade or above were used as received. All solutions were filtered through 0.45 μm cellulose membrane filters (Heshi Technology Development Co., Ltd., Tianjin, China) before CE analysis. Stock solutions of catecholamines (50 μM) were prepared by dissolving the compounds in 0.10 M HCl. These solutions were stored in dark at −20 °C and were diluted as required to obtain the working standards. FITC as the fluorescence tag for catecholamines was dissolved in acetone and was stored in dark at −20 °C before use. The BGE was prepared by dissolving 0.7627 g of $Na_2B_4O_7{\cdot}10H_2O$ and 2.2700 g of β-CD in 90 mL of purified water, adjusted to pH 9.5 with 0.1 M NaOH solution or 0.1 M HCl solution and then mixed with 10 mL of ACN.

2.2. Instrumentation

The home-built instrumental set-up was based on our previous work [21] with slight modifications, *i.e.*, two color filters were used in the detection system. Fig. 1 displays the schematic configuration of our CE-ICFO-LED-IFD system. In brief, a high-voltage supply (0–30 kV; Tianjin Dong Wen High Voltage Power Supply Plant, Tianjin, China) was used to drive the electrophoresis and an uncoated fused-silica capillary of 75 μm i.d. (Hebei Yongnian Optical Fiber, Hebei, China) was used as the separation capillary. A blue LED (Shifeng Optic and Electronics Co., Ltd., Shenzhen, China; power, 5 mW; peak wavelength, 480 nm; and spectral half-width, 25 nm) driven by a 15 V constant voltage source through a 500 Ω current-limiting resistor was employed as the excitation light source. The LED light beam was collimated by a double convex lens (GL12-006-010; Beijing Golden Way Scientific Co., Ltd., Beijing, China), passed through a bandpass filter (FF01-390/482/563/640-25; Semrock, Rochester, NY, USA), and focused on one end of a 20 cm × 40 μm optic fiber (Beijing Glass Institute, Beijing, China). The other end of the optical was inserted into the end of the separation capillary to introduce the exciting light beam to the sample detection window. The emitted fluorescence was captured by a 60× microscope objective with a spatial filter (Olympus, Japan), passed through a bandpass filter (FF01-446/523/600/677-25; Semrock), and detected by a R105UH PMT (Beijing Hamamatsu Photon Techniques, Beijing, China) detector. The output signal was recorded and processed with a personal computer.

In order to validate the CE-ICFO-LED-IFD methodology, the urine samples containing catecholamines were also simultaneously analyzed by an Agilent 1200 series HPLC system (Palo Alto, CA, USA) comprising a high-pressure G1311A gradient quaternary pump, an online G1322A vacuum degasser and a Rheodyne 7725i six-port valve with a 20 μL sample loop (Cotati, CA, USA) at a detection wavelength of 280 nm. Chromatographic separation was performed on a 150 × 4.6 mm i.d. Welchrom-C18 (5 μm) column (Welch Materials, Inc., Shanghai, China). The mobile phase was a solvent mixture of 0.14% sodium 1-heptanesulfonate (pH 3.0)-methanol (13:7). The pH was adjusted with concentrated phosphoric acid.

2.3. Sample preparation and derivatization with FITC

Urine samples (5.0–10 mL) were collected from five healthy volunteers and then derivatized with FITC without prior clean up procedures. In brief, 10 μL of DA, EP and NE standard or sample was mixed with 0.50 mL of 10 mM borate buffer (pH 9.5). FITC solution (in acetone) was added to get a final concentration 20 times greater than the concentration of DA, EP, NE or their total concentration. The solution was vortexed and kept at room temperature for 12 h. The derivatized samples were stored in dark at 4 °C before

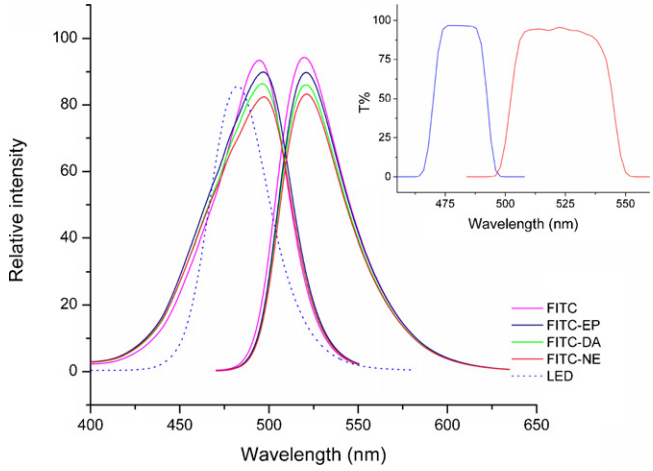

Fig. 2. Fluorescence excitation and emission spectra of free FITC, FITC-DA, FITC-EP and FITC-NE and emission spectrum of the blue LED. The inset displays the bandpass wavelengths of the filters for the excitation light source and emission light, respectively.

analysis. The FITC-tagged catecholamines were then separated and analyzed by the CE-ICFO-LED-IFD system under optimal CE conditions.

2.4. CE procedure

The capillary was treated by flushing with 1.0 M NaOH for 30 min before its first use. Between two consecutive injections, the capillary was rinsed sequentially with 0.10 M NaOH, water, and BGE for 3 min each. Samples were injected into capillary by hydrodynamic flow for 10 s at a height of 20 cm. Separations were performed at 13 kV using a BGE containing 10% v/v ACN, 20 mM β-CD and 20 mM sodium borate (pH 9.5). The total length of the capillary was 47 cm with an effective length of 45 cm. All CE procedures were conducted at room temperature.

3. Results and discussion

3.1. Choice of LED and spectral filter

Fig. 2 displays the fluorescence excitation and emission spectra of free FITC, FITC derivatized dopamine (FITC-DA), epinephrine (FITC-EP) and norepinphrine (FITC-NE). FITC, FITC-DA, FITC-EP and FITC-NE all show strong fluorescence at an excitation maximum of ~495 nm and an emission maximum of 525 nm. The Stokes' shift is only 30 nm. LEDs possess broader spectral bandwidth than lasers, typically 25 nm full width at half maximum versus 1 pm for lasers. If a LED with maximum emission wavelength at 495 nm is used, approximately 35% of the excitation light will overlap with the fluorescence emission of FITC-tagged catecholamines, resulting in high background. In other words, the LEDs' polychromaticity can lead to a high background signal since the scattered light from an LED coincides with the detection wavelength. Thus, both the excitation light from LED and fluorescence light from sample must be spectrally filtered. The longer wavelength band of LED has to be removed so that only the fluorescence emission signal of the sample is allowed to reach the PMT. The choice of filters must satisfy three requirements: the LED filter must match with the excitation spectrum of the analyte; the emission filter must match with the emission spectrum of the analyte; and finally, there should be minimal crosstalk between the filters and maximum fluorescent signal transmission. de Jong and Lucy [22] have already demonstrated that a bandpass filter positioned after LED can improve the signal-to-noise ratio (*S*/*N*). In another case, Yang et al. [23] reported that a bandpass filter at the excitation axis could obtain 40-fold enhancement of sensitivity as compared to no excitation filter design. For these reasons, a 482 nm bandpass filter and another 523 nm bandpass filter were chosen as the excitation and emission filters respectively since these spectral bands exactly match with the excitation and emission wavelengths of the FITC-tagged catecholamines. The inset of Fig. 2 displays the transmission spectra of the bandpass filters. The bandwidths of the two bandpass filters at 482 and 523 nm are nominally 90% transmittances over 18 and 42 nm, respectively. The 523 nm band perfectly matches with the emission spectra of FITC-tagged catecholamines whereas the 482 nm band matches with the emission spectrum of the blue LED. The crosstalk between the two bands is very small. These attributes are the keys to minimize background noise and improve the *S*/*N*.

3.2. Optimization of CE separation conditions

It is well-known that the structures of DA, EP and NE are very similar with all containing a dihydroxyphenyl and an amine moiety. In order to achieve efficient separation of DA, EP and NE, five major experimental conditions including concentrations of ACN and β-CD in BGE, concentration of borate buffer, BGE pH and applied voltage were carefully investigated.

Fig. 3A displays the effect of ACN (0.0–14% v/v) on the resolution of the FITC-tagged catecholamines. The migration follows the order: DA, EP and NE as shown in Fig. 4. The resolutions of DA/EP and EP/NE pairs increase with the increase of ACN concentration in the BGE (20 mM β-CD and 20 mM sodium borate at pH 9.5). The best resolution is at 10% v/v ACN. The addition of organic modifier to the BGE can change the system partition coefficient, viscosity, polarity and electroosmotic flow (EOF), thereby increasing selectivity, efficiency and resolution. However, further increase in ACN will decrease the resolution of DA/EP and EP/NE pairs. ACN in the BGE can affect the electrophoretic properties of the FITC labeled catecholamines in two aspects by: (1) lowering the EOF with the decrease in the viscosity and dielectric constant of BGE [24]; and (2) modifying the solubility of FITC labeled catecholamines in BGE and altering the formation constants of the β-CD-FITC labeled catecholamine inclusion-complexes [25,26]. Finally, the 10% v/v ACN was chosen as the optimal concentration in the BGE for subsequent experiments as it provides the best resolutions of the FITC labeled catecholamines.

The effect of β-CD concentration (5.0–25 mM) on the separation of DA, EP and NE was investigated and depicted in Fig. 3B. The resolutions of DA/EP and EP/NE pairs increase with the increase of β-CD concentration in the BGE (10% v/v ACN and 20 mM sodium borate at pH 9.5) until the concentration reaches 20 mM. β-CD is a cyclic oligomer composed of seven glucopyranose units linked by α (1,4) bonds and can form inclusion complexes with DA, EP and NE. Thus, the three inclusion complexes can be separated by their differences in molecular size, polarity and hydrophobic character. In this work, β-CD provides good separation ability for DA, EP and NE although its application in chiral separation is considered more significant. Cyclodextrins have been used extensively in separation science as they are known to discriminate positional isomers, functional groups, homologues and enantiomers [27,28]. The improvement in resolutions of DA/EP and EP/NE pairs in the presence of β-CD could be attributed to the formation of inclusion complexes between FITC labeled catecholamines and β-CD. The hydrophobic cavity of β-CD could be occupied to different extents by FITC-DA, FITC-EP and FITC-NE based on their steric structures, electrostatic interactions and van der Waals forces with β-CD, resulting in the modulation of their separation in CE. As such, 20 mM was chosen as the optimal concentration of β-CD in the BGE for separation of FITC-tagged catecholamines in this work.

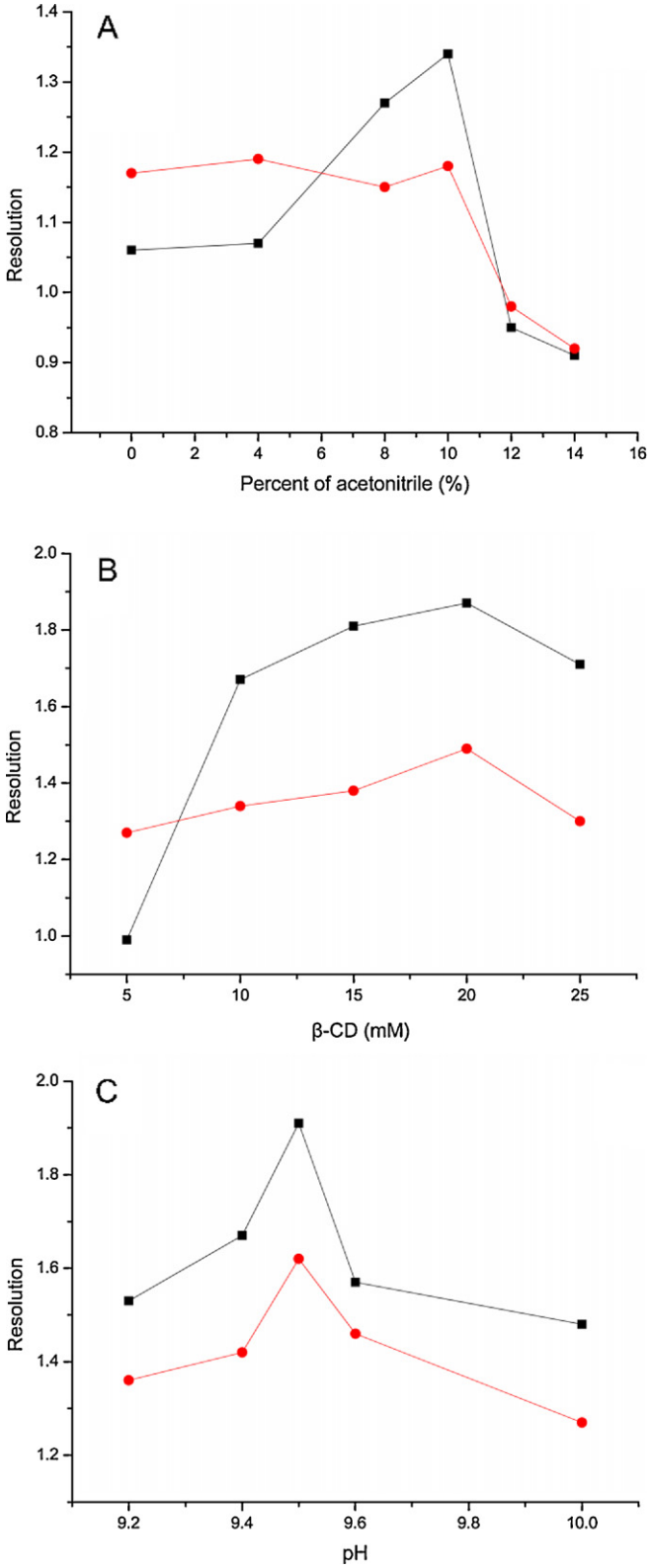

Fig. 3. Effect of (A) acetonitrile concentration, (B) β-CD concentration and (C) background electrolyte pH on the resolution of the FITC-tagged catecholamines. Dopamine and epinephrine pair (—■—) and epinephrine and norepinephrine pair (—●—).

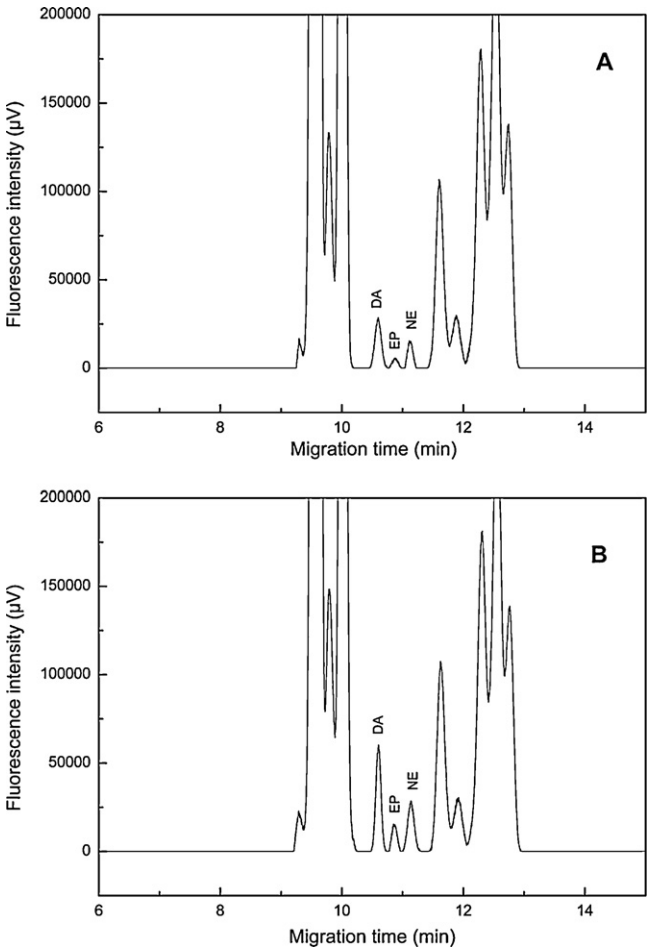

Fig. 4. Electrophoretic separation of urine sample derivatized by FITC. (A) urine sample and (B) urine sample spiked with 750 nM DA, 90 nM EP and 300 nM NE. The background electrolyte was 10% v/v acetonitrile, 20 mM β-CD and 20 mM borate (pH 9.5). The applied voltage was 13 kV.

Fig. 3C displays the effect of BGE pH (9.2–10.0) on the separation of DA, EP and NE. The resolutions of DA/EP and EP/NE pairs increase with the increase of pH in the BGE (10% v/v ACN, 20 mM β-CD and 20 mM sodium borate). The best resolution is at pH 9.5 and decreases with further increase in pH. When the pH was 10.0, the current reached 51 μA, producing excessive Joule heating. In addition, the effects of borate buffer concentration and applied voltage were also studied. It was found that 20 mM borate and an applied voltage of 13 kV produced the best separation of DA, EP and NE. In summary, the optimal CE conditions for separation of FITC-tagged catecholamines are BGE of 10% v/v ACN, 20 mM β-CD and 20 mM borate (pH 9.5) and an applied voltage of 13 kV.

3.3. *Linearity, limit of quantification, limit of detection and repeatability*

The linearity, limit of quantification (LOQ), limit of detection (LOD) and repeatability of the three catecholamines were assessed by using our CE-ICFO-LED-IFD system under the optimal CE conditions. The calibration curves were determined by plotting the peak area against concentration of analyte. The LOQ and LOD are the minimum analyte concentrations yielding an *S*/*N* of 10 and 3, respectively. Table 1 summarizes the analytical figures of merit of our CE-ICFO-LED-IFD system. The linear ranges are 3.0–500 nM for DA, 1.0–500 nM for EP and 3.0–500 nM for NE. The LOQs of DA, EP and NE are 3.5, 1.0 and 3.1 nM, and the LODs are 1.0, 0.3 and 0.9 nM,

Table 1
Coefficients of variation for migration time and peak area, and analytical figures of merit of the proposed CE-ICFO-LED-IFD method.

Catecholamines	DA	EP	NE
Coefficient of variation (%)[a] of			
Migration time	0.12	0.16	0.13
Peak area	2.0	1.6	1.8
Linear range (nM)	3.0–500	1.0–500	3.0–500
Regression equation[b]	$y = 1.91x - 0.97$	$y = 5.05x - 0.49$	$y = 2.30x - 1.29$
Correlation coefficient	0.9995	0.9997	0.9993
Limit of quantification (nM)	3.5	1.0	3.1
Limit of detection (nM)	1.0	0.3	0.9

[a] $n = 6$.
[b] x: concentration of FITC-tagged DA, EP or NE (μM) and y: fluorescence intensity.

Table 2
Determination of catecholamines in urine samples by HPLC and CE-ICFO-LED-IFD methods.

Urine sample	Analyte	Concentration[a](nM)	RSD[c] (%)	Concentration[b] (nM)	RSD[c] (%)
1	DA	672	2.09	665	3.56
	EP	69	2.36	76	3.07
	NE	238	2.55	231	3.82
2	DA	822	2.21	830	2.94
	EP	83	2.14	77	3.08
	NE	187	2.61	193	3.12

[a] Determined by the HPLC method.
[b] Determined by the CE-ICFO-LED-IFD method.
[c] Three replicates were performed.

respectively. Excellent reproducibility was obtained with the coefficients of variation ($n = 6$) for migration time and peak area less than 0.16 and 2.0%, respectively. The migration times are 10.61, 10.83 and 11.14 min for DA, EP and NE, respectively and the catecholamines are completely separated within 11.5 min as shown in Fig. 4. The LOQs obtained from our CE-ICFO-LED-IFD method are slightly better than that of the LC-MS/MS method which are 11.7, 1.47 and 15.8 nM for DA, EP and NE, respectively. Another advantage of our method is that it requires only FITC derivatization and no prior sample clean up procedures; however, the LC-MS/MS method requires an on-line solid phase extraction to clean-up the samples before analysis [4]. In essence, our CE-ICFO-LED-IFD method for determining catecholamines is relatively simple and sensitive.

3.4. Comparison with HPLC method

In order to verify the accuracy of the proposed method, the concentrations of DA, EP and NE in urine samples were simultaneously determined using a HPLC method in Chinese Pharmacopoeia [29]. Table 2 summarizes the results obtained with the HPLC method and our proposed method and they are in good agreement, demonstrating that our proposed method is reliable.

3.5. Sample analysis

Our proposed method was applied to determine catecholamines in urine samples from five healthy human subjects. The samples were derivatized with FITC and then subjected to CE-ICFO-LED-IFD analysis. A typical electropherogram from CE separation of a sample is depicted in Fig. 4A. The electropherogram shows many peaks and some of them are not identified. To verify the DA, EP and NE peaks, 750 nM of DA, 90 nM of EP and 300 nM of NE were added to the sample and analyzed again as shown in Fig. 4B. It clearly indicates that the DA, EP and NE peaks are well resolved with no interference from other unknown compounds in the urine matrix under the optimal CE conditions. DA migrates faster than EP and NE; and NE migrates the slowest. The concentrations of DA, EP and NE in the urine samples and the recovery tests are summarized in Table 3. When the concentrations of catecholamines in the samples are too high, appropriate dilutions of the FITC derivatized samples with

Table 3
Determination and recovery of catecholamines in urine samples using the proposed CE-ICFO-LED-IFD system.

Urine sample	Analyte	Initial (nM)	RSD[a] (%)	Added (nM)	Found (nM)	Recovery (%)	RSD[a] (%)
1	DA	755	3.47	750	1553	106	2.58
	EP	60	4.02	90	151	101	2.96
	NE	350	3.39	300	648	99.3	3.29
2	DA	589	3.18	750	1336	99.6	3.62
	EP	84	3.95	90	177	103	4.20
	NE	275	4.27	300	578	101	3.24
3	DA	940	3.69	750	1702	102	2.48
	EP	53	3.26	90	140	96.7	3.13
	NE	289	3.52	300	586	99.0	2.84
4	DA	803	3.44	750	1557	101	4.57
	EP	65	3.86	90	158	103	4.03
	NE	323	4.11	300	611	96.0	3.94
5	DA	947	2.89	750	1719	103	2.88
	EP	34	3.77	90	122	97.8	3.51
	NE	193	3.31	300	488	98.3	3.06

[a] Three replicates were performed.

BGE are required before CE analysis. The recoveries are satisfactory with 99.6–106% for DA, 96.7–103% for EP and 96.0–101% for NE, indicating that our novel CE technique can provide reliable analysis of catecholamines in real sample.

4. Conclusion

An accurate and simple CE method coupled with in-column optical fiber LED-induced fluorescence detection for quantification of free catecholamines in urine has been successfully developed. The use of bandpass filter to select an appropriate excitation light of the LED enables our CE system to achieve low background and detection limit. It is clear that the proposed method is useful for simultaneous analysis of DA, EP and NE in urine samples without complex pretreatment procedures. Thus, the present method holds great potential in the areas of clinical diagnosis and treatment of catecholamine-producing tumors. Although the sensitivity of detection is not as good as LIF detection, our proposed equipment is more cost-effective and compact. Currently we are exploring to apply multi-LED or LED-array coupled to fluorescence detector to enhance the sensitivity of detection. It is anticipated that the proposed methodology can be applied to simultaneous determination of catecholamines in biological samples with much lower concentrations.

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References

[1] B. Kagedal, D.S. Goldstein, J. Chromatogr. B 429 (1988) 177.
[2] R.T. Peaston, C. Weinkove, Ann. Clin. Biochem. 41 (2004) 17.
[3] R.P.H. Nikolajsen, A.M. Hansen, Anal. Chim. Acta 449 (2001) 1.
[4] W.H.A. de Jong, E.G.E. de Vries, B.H.R. Wolffenbuttel, I.P. Kema, J. Chromatogr. B 878 (2010) 1506.
[5] Q. Gu, X.Z. Shi, P.Y. Yin, P. Gao, X. Lu, G.W. Xu, Anal. Chim. Acta 609 (2008) 192.
[6] V. Carrera, E. Sabater, E. Vilanova, M.A. Sogorb, J. Chromatogr. B 847 (2007) 88.
[7] W.L. Tseng, S.M. Chen, C.Y. Hsu, M.M. Hsieh, Anal. Chim. Acta 613 (2008) 108.
[8] C.E. Lin, H.T. Cheng, I.J. Fang, Y.C. Liu, C.M. Kuo, W.Y. Lin, C.H. Lin, Electrophoresis 27 (2006) 3443.
[9] P. Britz-McKibbin, D.D.Y. Chen, Anal. Chem. 72 (2000) 1242.
[10] M. Chicharro, A. Sanchez, A. Zapardiel, M.D. Rubianes, G. Rivas, Anal. Chim. Acta 523 (2004) 185.
[11] O. Klett, I. Nischang, L. Nyholm, Electrophoresis 23 (2002) 3678.
[12] R.A. Wallingford, A.G. Ewing, Anal. Chem. 60 (1988) 258.
[13] Y.M. Liu, C.Q. Wang, H.B. Mu, J.T. Cao, Y.L. Zheng, Electrophoresis 28 (2007) 1937.
[14] Y.G. Hu, X.X. Li, Z.T. Pang, J. Chromatogr. A 1091 (2005) 194.
[15] R.H. Zhu, W.T. Kok, Anal. Chem. 69 (1997) 4010.
[16] M. Du, V. Flanigan, Y.F. Ma, Electrophoresis 25 (2004) 1496.
[17] F. Robert, L. Bert, L. Denoroy, B. Renaud, Anal. Chem. 67 (1995) 1838.
[18] Z.D. Peterson, D.C. Collins, C.R. Bowerbank, M.L. Lee, S.W. Graves, J. Chromatogr. B 776 (2002) 221.
[19] K. Vuorensola, J. Kokkonen, H. Siren, R.A. Ketola, Electrophoresis 22 (2001) 4347.
[20] D. Xiao, L. Yan, H.Y. Yuan, S.L. Zhao, X.P. Yang, M.M.F. Choi, Electrophoresis 30 (2009) 189.
[21] S.L. Zhao, H.Y. Yuan, D. Xiao, Electrophoresis 27 (2006) 461.
[22] E.P. de Jong, C.A. Lucy, Anal. Chim. Acta 546 (2005) 37.
[23] B.C. Yang, H.Z. Tian, J. Xu, Y.F. Guan, Talanta 69 (2006) 996.
[24] C.W. Huie, Electrophoresis 24 (2003) 1508.
[25] M. Taghvaei, G.H. Stewart, Anal. Chem. 63 (1991) 1902.
[26] S. Fanali, J. Chromatogr. A 875 (2000) 89.
[27] E. Schneiderman, A.M. Stalcup, J. Chromatogr. B 745 (2000) 83.
[28] J. Mosinger, V. Tomankova, I. Nemcova, J. Zyka, Anal. Lett. 34 (2001) 1979.
[29] Committee of Chinese Pharmacopoeia, Chinese Pharmacopoeia (Part II), Chemical Industry Press, Beijing, 2005, p. 334.