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A validated capillary electrophoretic method for the determination of indacaterol and its application to a pharmaceutical preparation



Tufan Güray^{a,*}, Muzaffer Tunçel^b, Ulku Dilek Uysal^c

^a Department of Chemistry, Faculty of Arts and Science, Eskisehir Osmangazi University, 26480, Eskisehir, Turkey

^b Lefke European University, Faculty of Pharmacy, Department of Analytical Chemistry, Lefka, Cyprus

^c Department of Chemistry, Faculty of Science, Anadolu University, 26470, Eskişehir, Turkey

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ABSTRACT

Indacaterol is a new inhaled ultra-long acting β_2 -agonist. It has been recently approved in the European Union for the treatment of chronic obstructive pulmonary disease. This paper reports, for the first time, a method for the determination and validation of Indacaterol (IND) using an internal standard in capsules. Capillary electrophoretic separation was performed on an uncoated fused-silica capillary (50 cm effective length, 75 μm i.d.) and background electrolyte composed of 20 mmol L^{-1} of sodium tetraborate buffer, 15% (v/v) methanol (pH = 10.0) with the application of 20 kV of potential; 10 s at $5 \times 10^3 \text{ N m}^{-2}$ (50 mbar) of injection time; and wavelength of 200 nm and 25 °C of temperature. The linearity was evaluated in the range of $4.90 \times 10^{-6} \text{ mol L}^{-1}$ (2.50 $\mu\text{g mL}^{-1}$) and $3.94 \times 10^{-5} \text{ mol L}^{-1}$ (20.00 $\mu\text{g mL}^{-1}$), with $R = 0.9993$ for inter-day. LOD and LOQ values were $2.18 \times 10^{-8} \text{ mol L}^{-1}$ (0.011 $\mu\text{g mL}^{-1}$) and $7.25 \times 10^{-8} \text{ mol L}^{-1}$ (0.037 $\mu\text{g mL}^{-1}$) for inter-day, respectively. The precision values were 0.50–1.06% for intra-day and 2.12% for inter-day as RSD%. The accuracy was tested by the standard addition method with the recovery values being between 98.79 and 99.09 as percentages with RSD% interval of 0.01–0.80. The developed method was validated according to ICH guidelines. Indacaterol was successfully determined in Arcapta[®] capsule dosage form by the validated CE method with a relative error of 0.28%. The result was within the requirements of the USP 34-NF29. Therefore, the validated method may be used for the determination of Indacaterol in its capsules in quality control laboratories.

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1. Introduction

Indacaterol (IND) 5-((1R)-2-[(5,6-diethyl-2,3-dihydro-1H-inden-2-yl)amino]-1-hydroxyethyl)-8-hydroxy-2(1H)-quinolinone male-

ate (Fig. 1) is a new, once daily orally inhaled, pre-metered single-unit dose capsule-based dry powder ultra-long acting β_2 -agonist which has been recently approved in the European Union for keeping the airways open in adults with chronic obstructive pulmonary disease (COPD) [1]. It has also recently been approved

* Corresponding author.

E-mail addresses: tguray@ogu.edu.tr, tufanguray@gmail.com (T. Güray).

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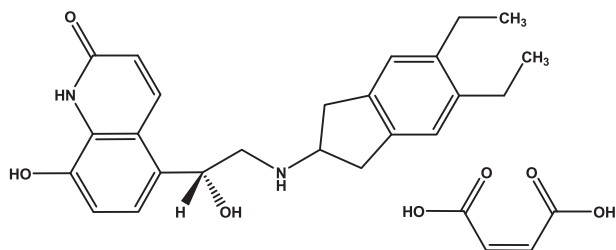


Fig. 1 – The chemical structure of IND.

for COPD by the U. S. Food and Drug Administration (FDA) [2]. COPD is a long-term disease where the airways and air sacs inside the lungs become damaged or blocked, leading to difficulty breathing air in and out of the lungs [3]. Certain drug combinations, such as indacaterol/glycopyrronium dose combination, have also been used for the symptomatic control of COPD in adults. It should be emphasized that local prescribing information should be sought for details of contraindications, special warnings and precautions relating to the use of these kinds of combinations [4]. As a consequence, analytical methods are required for both supporting indacaterol's pharmacological properties, therapeutical efficacy and tolerability studies [4–6] and for an understanding of these combinations. Currently, a number of publications have been reported for the determination of IND in pharmaceuticals [7,8] and other samples [9–13]. The methods employed are spectrophotometry and fluorometry [8,14,15] and HPLC [16] in pharmaceutical preparations, HPLC-MS in human plasma, serum or urine for the determination of IND [9,10,17].

Capillary electrophoresis (CE) is a relatively new and extremely powerful technique. It offers numerous advantages over conventional chromatographic methods because of its unique separation mechanism, speed, higher efficiency, versatility, environmental friendliness, resources utilization, small sample amount, not requiring further purification, and permitting several kinds of buffers and additives usage as the electrolyte [18,19]. Moreover, the sensitivity of CE is generally better than that of HPLC in UV absorbance detection due to the short path length of CE [18,20]. CE has many applications in biochemistry, pharmaceutical science [21], bioscience [22], ion analysis [23], food analysis [24–26] and environmental science [23] fields. To the best of our knowledge, no other publication has addressed the determination of IND by capillary electrophoresis (CE) or capillary zone electrophoresis (CZE). Here we describe the new environmental-friendly, fully validated CE method for the determination of IND in pharmaceutical formulation.

2. Materials and methods

2.1. Chemicals, reagents and samples

HPLC grade methyl paraben (Internal standard, IS), methanol (MeOH), and acetonitrile (ACN) were purchased from Sigma–Aldrich (St. Louis, MO, USA). ACS grade sodium tetraborate and sodium phosphate were purchased from Merck GmbH

(Darmstadt, G). Indacaterol maleate (IND) was purchased from Santa Cruz (Santa Cruz Biotechnology, Inc., 10410 Finnell St., Dallas, USA). Ultrapure water with a resistivity of $18.2 \mu\text{S cm}^{-1}$ (Millipore, Molsheim, France) was used throughout this work. Arcapta[®] was supplied from Novartis (Novartis Pharma Stein AG, Switzerland).

2.2. Apparatus

All CE separations were conducted on a Capillary Electrophoresis 1600 system with diode array UV detector (Agilent Technologies, G1600 A, Oregon, USA). Electrophoresis was performed in fused silica capillaries of $75 \mu\text{m}$ i.d. and of effective length of 50 cm and total 57 cm long (Agilent). Gas bubbles from all solutions and samples were removed by ultrasonic bath Sonorex (Bandelin, Berlin, G) and they were then centrifuged in a 4000 rpm speed centrifuge (Sigma, 1-6P Laboratory Centrifuge). The solutions' pH was measured using a model pH/ion meter- 720A with an Orion 71-03 glass electrode (ThermoOrion Beverly, MA 01915-6199, USA). All of the buffers and sample solutions were filtered through a regenerated cellulose (RC) membrane filter $0.45 \mu\text{m}$ prior to analysis (La-Pha-Pack, Rockwood, TN, USA).

2.3. Solution and sample preparation

A stock solution ($4.92 \times 10^{-4} \text{mol L}^{-1}$) was prepared by dissolving 25 mg of IND in 100 mL MeOH. Serial dilutions were performed with the 10% (v/v) MeOH/water mixture to obtain the appropriate concentration range. The standard solutions were stable for at least two weeks if kept in a refrigerator at $+4 \text{ }^\circ\text{C}$.

15.2 mg methyl paraben (IS) was dissolved in 30 mL of MeOH and was then diluted with ultrapure water up to 100 mL.

A stock sodium tetraborate buffer solution (100 mmol L^{-1}) was prepared, and then relevant running buffer solutions were obtained from this solution. The pH of the solution was adjusted to a desired value with $0.1 \text{ mol L}^{-1} \text{HCl}$ or $0.1 \text{ mol L}^{-1} \text{NaOH}$. This was prepared daily by mixing appropriate volumes of stock buffer solutions, water and MeOH in order to adjust the pH to the desired value.

Each inhalation powder hard capsule (Arcapta[®]) contains 194 μg of indacaterol maleate, equivalent to 150 μg of indacaterol and certain inactive ingredients, such as lactose monohydrate and gelatine. Ten capsules were accurately weighed individually taking care to preserve the identity of each capsule. The contents of each capsule were removed. The emptied shells were then individually accurately weighed, and the net weight of contents for each capsule was calculated by subtracting the weight of shell from each respective gross weight. The drug substance content of each capsule was calculated from the net weight of the individual capsule content. The drug substance content of each capsule (25.3 mg of Arcapta[®]) was transferred into a small flask and extracted with 3 mL of MeOH. The mixture was centrifuged at 4000 rpm for 5 min and then filtered through a $0.45 \mu\text{m}$ membrane filter (RC). A 0.1 mL aliquid of the sample solution was diluted to 1 mL with water. Then 30 μL of IS at $1.05 \times 10^{-3} \text{mol L}^{-1}$ was added. Finally, it was injected through the CE capillary.

2.4. CE conditions

The system was thermostated at 25 °C and the new capillary was conditioned by flushing at $9.35 \times 10^4 \text{ N m}^{-2}$, sequentially with 1.0 mol L^{-1} NaOH for 15 min, with 0.1 mol L^{-1} NaOH for 15 min, water for 15 min and running buffer for 15 min. Between two consecutive analyses, the capillary was flushed sequentially with distilled water for 2 min, 0.1 mol L^{-1} NaOH for 1 min, and distilled water for 3 min, and finally with a running buffer for 5 min prior to use. All buffers and sample solutions were filtered through a $0.45 \mu\text{m}$ membrane filter prior to the analyses. Before injection through the capillary of CE, all solutions and samples were sonicated for 5 min. These were injected through the capillary for 10 s using a hydrodynamic injection mode applying low-pressure $5 \times 10^3 \text{ N m}^{-2}$ from the anodic side. Signals were detected at 200 nm and the migration times were 4.84 and 6.47 min for IND and IS, respectively.

2.5. Method validation

The method was validated according to the International Conference on Harmonization (ICH) [27] guidelines for validation of analytical procedures.

The results were evaluated using a rate of peak normalization (rPN) calculated by dividing the peak normalization value of IND into the peak normalization of IS [$rPN = PN_{\text{IND}}/PN_{\text{IS}}$]. The peak normalization values for IND and IS were found by dividing their individual areas into their retention times IND [$PN_{\text{IND}} = \text{Peak area}_{\text{IND}}/\text{Retention time}_{\text{IND}}$] and IS [$PN_{\text{IS}} = \text{Peak area}_{\text{IS}}/\text{Retention time}_{\text{IS}}$]. During the experimental evaluations, the related parameters were investigated against PN or rPN.

2.5.1. System suitability of the method

The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. The parameters were tested for basic analytical parameters including capacity factor, resolution, tailing of the peak, theoretical plate, retention time and percentage of repeatability and were evaluated using Agilent Software for system suitability of the developed method.

2.5.2. Precision

The validation of tests for assay and for quantitative determination of impurities includes an investigation of precision. The precision of the method was calculated by repeatability (intra-day) and intermediate precision (inter-day). Repeatability was evaluated by assaying the samples of the same concentrations for five times over three successive days ($n = 6$). Standard solution of IND ($0.98 \times 10^{-5} \text{ mol L}^{-1}$) and constant IS solution ($3.06 \times 10^{-5} \text{ mol L}^{-1}$) were used for the precision experiments. Intermediate precision was found by comparing the experiments on three different days.

2.5.3. Calibration tests

Calibration studies were carried out by preparing three sets and five dilutions ($n = 5$) for each set in the range of $4.96 \times 10^{-6} \text{ mol L}^{-1}$ and $3.94 \times 10^{-5} \text{ mol L}^{-1}$. These were then

injected through the capillary consecutively for three days (intra-days). Calibration curves were found by linear regression analysis using the least square method. Slope, intercept, correlation coefficient, standard deviation of regression equation for the calibration equations, and confidence limits (for $P < 0.05$) were calculated both intra-day and inter-day. Certain analytical parameters, such as LOD and LOQ values, were found by computing the integrated peak from the CE electropherogram. LOD and LOQ values were calculated [(standard deviation of regression equation)/(slope of regression equation)] by multiplying by 3.3 and 10, respectively.

2.5.4. Accuracy of the method

The accuracy of the method was tested by the standard addition method as stated: Definite amounts of standard IND (0.598 mL; 1.201 mL and 1.799 mL of $4.92 \times 10^{-4} \text{ mol L}^{-1}$) were spiked into approximately 25.3 mg of Arcapta®. It was extracted with 3 mL of MeOH. The mixture was centrifuged at 4000 rpm for 5 min and then filtered through a $0.45 \mu\text{m}$ membrane filter (RC). An aliquot of 0.1 mL of the sample solution was diluted to 1 mL with water (Final concentrations were 8.18×10^{-6} , 1.41×10^{-5} , and $1.84 \times 10^{-5} \text{ M}$, respectively) and then 30 μL of IS at $1.05 \times 10^{-3} \text{ mol L}^{-1}$ was added. It was then injected through the CE capillary.

2.6. Determination of IND in pharmaceutical formulation

IND was determined in the pharmaceutical formulation of Arcapta® inhalation powder hard capsule dosage form by the developed CE method. The sample was weighted and prepared as in the section 'Solution and Sample Preparation'.

3. Results and discussion

3.1. Optimization of the method

IND has two pK_a values of 8.5 and 9.7 [16]. Even if EOF is still strong at pH 7.5 and higher pH values, IND moves with EO peak at 7.5. At higher pH of 7.5, IND moves after EOF. The best peak shape was observed at pH 10.0 regarding efficiency and peak width. Retention time of IND was around the same at pH 8, 9, 9.5, 10.0 and 10.5. At pH 10.5, the IND peak was broadened. At pH 10.0, the value of rPN is the highest (Fig. 2). Thus, pH 10 buffer was selected as an optimum pH value. IND is completely in anionic form around pH 10.

Sodium phosphate and sodium tetraborate buffers were tried as alkaline buffer. Sodium phosphate was not used due to high current, and a high noise signal in the electropherograms. In further optimization studies, sodium tetraborate buffer was used as the running buffer in this study.

In the CE analysis, the addition of organic modifier to the running buffer changes both the zeta potential and electrolyte viscosity. The organic modifier can change selectivity by changing electroosmotic flow mobility. For this purpose, methanol and acetonitrile as organic modifiers for the running buffer, consisting of sodium tetraborate, were tried. Since the IND solution was not dissolved entirely in acetonitrile, the standard solutions of IND and the sample were

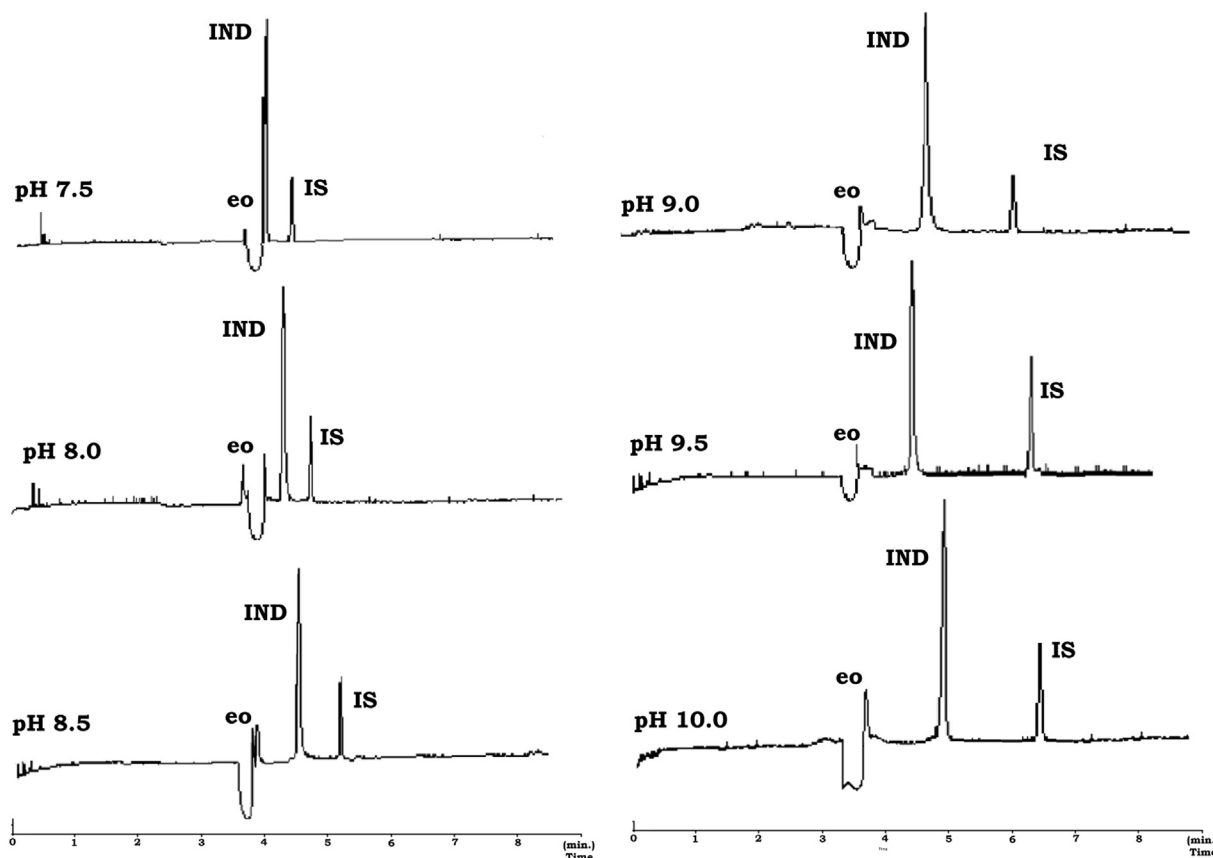


Fig. 2 – The electropherograms of IND ($4.92 \times 10^{-5} \text{ mol L}^{-1}$) and IS ($3.06 \times 10^{-5} \text{ mol L}^{-1}$) in the employment of 20 mmol L^{-1} sodium tetraborate buffer, 15% (v/v) MeOH, at different pH values in the use of 20 kV of applied potential, 10 s at $5 \times 10^3 \text{ N m}^{-2}$ of injection time, 200 nm of wavelength and 25°C of fixed temperature.

prepared by dissolving them in methanol and this was used as an organic modifier throughout the study.

The concentrations of sodium tetraborate of ($10\text{--}25$) mmol L^{-1} , percentages of organic modifier MeOH of $10\text{--}20$, % and pH ranges of $9.5\text{--}10.5$, applied potentials of $10\text{--}25$ kV, injection times on $5\text{--}10$ s at $5 \times 10^3 \text{ N m}^{-2}$ and the effect of wavelength (between 200 nm and 260 nm) were examined to determine the optimum conditions. Optimal conditions were determined in the viewpoint of peak shape, resolution, sensitivity and retention time. These were a running buffer consisting of 20 mmol L^{-1} sodium tetraborate solution, 15% (v/v) MeOH at pH = 10.0, 20 kV of applied potential, 10 s at $5 \times 10^3 \text{ N m}^{-2}$ injection time, 200 nm wavelength and a 25°C fixed temperature. Certain internal standards were tried to determine a suitable IS to increase the repeatability and sensitivity of the developed method. Nicotine amid, phenobarbital, methyl paraben, ethyl paraben, propyl paraben and butyl paraben were tried as the candidate for internal standard. Methyl paraben was a suitable IS for this system and appeared in a reasonable time.

The electropherogram of standard IND ($0.98 \times 10^{-5} \text{ mol L}^{-1}$) and IS ($3.06 \times 10^{-5} \text{ mol L}^{-1}$) solution in the optimum conditions is demonstrated in Fig. 2.

Under optimum conditions, IND and IS migrated in 4.84 (RSD 0.39%) and 6.47 (0.42%) minutes, respectively. The mean electrophoretic mobility toward the anode (m^2/sV) of IND and

IS was calculated as -1.63×10^{-4} (RSD: 0.98%) and -2.89×10^{-4} (RSD: 1.99%), respectively ($n = 3$).

3.2. Method validation

The solution containing $0.98 \times 10^{-5} \text{ mol L}^{-1}$ IND and IS ($3.06 \times 10^{-5} \text{ mol L}^{-1}$) was consecutively injected through the capillary to determine efficiency. Under optimum conditions, the obtained system suitability parameters are given in Table 1. The system suitability values confirm that the determination of IND can be achieved successfully.

The experimental values obtained from the results of examination of precision for IND are presented in Table 2.

As can be seen from Table 2, RSD values of 0.91–1.18% show good intra-day precision. Inter-day results were calculated from the three days of inter-day experiments obtaining on an RSD value of 1.04%. The RSD% values show that the method is precise and acceptable from an analytical point of view.

The calibration curve is given for IND in the range of $0.49 \times 10^{-5} \text{ mol L}^{-1}$ ($2.50 \mu\text{g mL}^{-1}$) and $3.94 \times 10^{-5} \text{ mol L}^{-1}$ ($20.0 \mu\text{g mL}^{-1}$) with $R = 0.9993$ for inter-day and, LOD and LOQ values in Table 3. As can be seen from Table 3, the calibration equation is linear ($r_{\text{P}} = 7.55 \times 10^5 C_{\text{IND}} - 0.11$) on the basis of inter-day results, with a good correlation coefficient (0.9993). The LOD and LOQ values were calculated to be

Table 1 – System suitability parameters of IND in the optimum conditions.

Parameters	Observed value (IND)	Observed value (IS)
Migration time (t as min)	4.84	6.47
Capacity factor (k')	0.33	0.77
Tailing factor (T)	1.23	1.41
Theoretical plates (N)	2.01×10^5	2.51×10^5
Resolution (R _s)	34.35	
Separation factor (α)	1.34	

Table 2 – The results of intraday and inter-day precision of IND (employing 0.98×10^{-5} mol L⁻¹ IND and 3.06×10^{-5} mol L⁻¹ IS) computed by the rPN vs concentration of IND (rPN = PN_{IND}/PN_{IS}).

	I. Day (n = 6) ^a	II. Day (n = 6) ^a	III. Day (n = 6) ^a	Inter-day (n = 18) ^a
\bar{X} ^b	0.60	0.59	0.59	0.59
s ^c	0.01	0.01	0.01	0.01
RSD% ^d	1.18	1.06	0.91	1.04
CL ^e	0.01	0.01	0.01	0.01

^a n is the number of experiments.

^b \bar{X} is the mean ratio of peak-normalization.

^c s is the standard deviation of the mean response.

^d RSD% is the relative standard deviation as percent.

^e CL is confidence limits, $\left(\frac{ts}{\sqrt{n}}\right)$.

2.18×10^{-8} mol L⁻¹ (0.011 μg mL⁻¹) and 7.25×10^{-8} mol L⁻¹ (0.037 μg mL⁻¹) for IND, respectively, on an inter-day basis. The results are reasonably low for LOD and LOQ for the determination of IND.

The accuracy of the method was examined by the standard addition method as stated in the experimental section of 'Accuracy of the Method'. The results are presented in Table 4.

Table 3 – Calibration elements for IND in the range of 0.49×10^{-5} mol L⁻¹ (2.5 μg mL⁻¹) and 3.94×10^{-5} mol L⁻¹ (20.0 μg mL⁻¹) in optimum condition.

	I. Day, n = 6	II. Day, n = 6	III. Day, n = 6	Inter-day, n = 18
m ^a	7.62×10^5	7.45×10^5	7.59×10^5	7.55×10^5
n ^b	-0.12	-0.11	-0.11	-0.11
R ^c	0.9991	0.9994	0.9991	0.9993
s _m ^d	0.04	0.03	0.04	0.03
CL ^e	±1456	±1202	±1442	±1290
LOD ^f (mol L ⁻¹)	2.16×10^{-8}	2.21×10^{-8}	2.16×10^{-8}	2.18×10^{-8}
LOQ ^g (mol L ⁻¹)	7.19×10^{-8}	7.36×10^{-8}	7.21×10^{-8}	7.25×10^{-8}

^a m is slope.

^b n is intercept.

^c R is correlation coefficient.

^d s_m is the standard deviation of calibration curve, $\left(s_m = \sqrt{\frac{s_r^2}{s_{xx}}}\right)$, s_r is standard deviation of regression.

^e CL is confidence limits, $\left(\frac{ts_m}{\sqrt{n}}\right)$.

^f LOD is limit of detection.

^g LOQ is limit of quantification.

Table 4 – The results of accuracy of IND (n = 6) by the standard addition method as stated in 'Accuracy of the Method' of the experimental section.

Added IND, (mol L ⁻¹)	Found IND, (mol L ⁻¹)	Recovery% (RSD%)
0.98×10^{-5}	0.97×10^{-5}	99.09 (0.21)
1.97×10^{-5}	1.94×10^{-5}	98.84 (0.01)
2.95×10^{-5}	2.91×10^{-5}	98.79 (0.80)

The calculated recoveries % (98.79–99.09%) demonstrate that the proposed method has excellent accuracy (Table 4). The recovery ranges % were in agreement with accepted criteria, which were in the range of 85–115%.

The ability of the method to remain unaffected by small but deliberate variations in the optimization parameters was measured via robustness to test the method's reliability during usage. In the robustness experimental design, a single variable has been changed at a time. For this purpose, certain parameters such as pH, sodium tetraborate concentration, MeOH percentage, applied voltage, wavelength, column temperature and injection time has been changed and the results have been compared than those in optimum conditions. The parameters concerning robustness are given in Supplementary Table 1. The values of standard error of the mean (SE < 1) for robustness parameters demonstrate that the developed method is highly reliable. All of the small changes are in an acceptable range regarding RSD% value < 2 for each parameter and it can be seen that the method is highly robust.

3.3. Application of the method to IND in the pharmaceutical preparation

The analysis of the IND in Arcapta[®] capsule dosage form was realized as stated in the experimental section of 'Preparation of Sample' and 'Determination of IND in pharmaceutical formulation'. A typical electropherogram of the Arcapta[®] capsule dosage form has been shown in Fig. 3.

The output of the experiments was evaluated and rPN values were calculated. The active substance, IND, was computed from the calibration equation. The results and evaluations are shown in Table 5. As can be seen from Table 5, the determined IND amount was 193.45 μg of indacaterol maleate with a relative error of 0.28% (certified IND value: 194 μg of indacaterol maleate equivalent to 150 μg of indacaterol) in Arcapta[®] capsule dosage form. Since IND is a new drug, any monograph in the pharmacopeias has not yet been reported. The acceptance criteria allows for a 15% deviation. The deviation of our result for IND (0.28%) is within limits. The relative standard deviation (RSD%) of the drug substance in the final dosage units should not be more than 2% according to the Pharmacopeia [28]. The RSD% value (0.37) in our study is smaller than this value (2%) (Table 5). As a result, the RSD% and the absolute error values are within limits. These considerations show us that the presented method is valid and reliable.

Supplementary Table 2 summarizes the analytical performance of our CE method compared to other methods for the determination of IND. Among these methods, HPLC-MS provides the lowest LOD as well as fluorescence for IND.

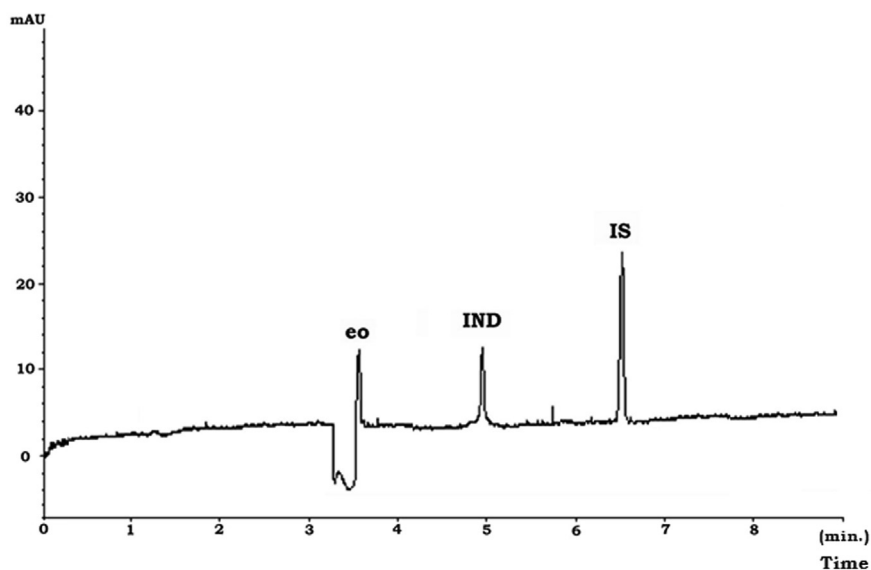


Fig. 3 – A typical electropherogram of Arcapta® capsule on optimum conditions.

Table 5 – The results of IND in Arcapta® inhalation powder hard capsule by the CE method (Certified value: 194 µg of indacaterol maleate equivalent to 150 µg of indacaterol on each capsule Arcapta®), (n = 6).^a

Parameters	Found (Relative Error, %) ^b (µg capsule ⁻¹)
\bar{X} ^c	193.45 (0.28%)
S ^d	0.72
RSD% ^e	0.37
CL ^f	0.72

^a n is the number of experiments.

^b Relative error is the magnitude of the difference between the exact value and the found value divided by the magnitude of the exact value as percent.

^c \bar{X} is the mean by regarding ratio of peak-normalization.

^d s is the standard deviation of the mean response.

^e RSD% is the relative standard deviation as percent.

^f CL is confidence limits, $\left(\frac{ts}{\sqrt{n}}\right)$.

Unfortunately, the method is expensive, not portable, requires an experienced technician, and has a matrix effect. Our CE method is comparable with the HPLC- Fluorescence method. HPLC-UV and UV-Vis. methods possess higher LOD values than those for our method.

4. Conclusion

A rapid, accurate, selective, reliable and environmentally friendly new capillary electrophoretic method for the determination of IND was developed and is validated in this study. The method is simple, easy to apply and IND was determined within 8 min. The method is also cheap and has many advantages over conventional chromatographic techniques, including reduction in the use of organic solvents, small

sample volume, and increased efficiency and resolution. To the best of our knowledge, this is the first CE study concerning the determination of IND.

The method has been validated with respect to precision, linearity range and accuracy, LOD, LOQ, specificity and robustness. All the system suitability parameters gave good results. The proposed method has been successfully applied to the analysis of IND in the pharmaceutical preparation of Arcapta® capsule dosage form.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jfda.2017.08.002>.

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