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Development and validation of an analytical method for the separation and determination of major bioactive curcuminoids in *Curcuma longa* rhizomes and herbal products using non-aqueous capillary electrophoresis

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ABSTRACT

A simple, fast and efficient non-aqueous capillary electrophoresis method (NACE) was developed for the simultaneous determination of three major bioactive curcuminoids (CMNs) in *Curcuma longa* rhizomes and its herbal products. Good separation, resolution and reproducibility were achieved with the background electrolyte (BGE) consisting a mixture of 15.0 mM sodium tetraborate and 7.4 mM sodium hydroxide (NaOH) in 2:10:15 (v/v/v) of water, 1-propanol, and methanol. The influences of background electrolyte, sodium hydroxide, water, sodium dodecyl sulfate and hydroxylpropyl-β-cyclodextrin on separations were investigated. The separation was carried out in a fused-silica capillary tube with reverse polarity. Hydrodynamic injection of 25 mbar for 12 s was used for injecting samples and a voltage of 28 kV was applied for separation. The ultrasonication method was used for the extraction of CMNs from the turmeric herbal products and the extract was filtered and directly injected without any further treatments. The limits of detection and quantification were less than 5.0 and 14.6 μg/ml respectively for all CMNs. The percentage recoveries for CMNs were $>97.2\%$ (%RSD, <2.62). The results obtained by the method were compared with existing spectrophotometric and HPLC methods. The related compounds in the extract did not interfere in the determination of CMNs. The proposed NACE method is better than existing chromatographic and electrophoretic methods in terms of simple electrophoretic medium, fast analysis and good resolution.

1. Introduction

Curcuma longa (turmeric) is one of the most extensively investigated medicinal plant species. In recent years, turmeric has received much attention worldwide due to its wide spectrum of pharmacological activities [1–4]. The plant is widely cultivated [5] and easily available to everyone, especially for rural populations for treatment of various health problems. Curcumin (CN), desmethoxycurcumin (BN) and bisdesmethoxycurcumin (DN) are the major bioactive compounds (Fig. 1) in turmeric rhizomes and are called as curcuminoids (CMNs). Research studies indicate that CN, BN and DN showed that they have different pharmacological activities. CN, the major constituent of CMNs, has relatively higher pharmacological activity including antioxidant, antibacterial and anticancer properties than other CMNs [6,7]. India is the largest producer and consumer of turmeric rhizomes. The turmeric extract or the rhizome powder is also widely used in traditional system of medicine in many countries [8,9]. Different varieties of *C. longa* are cultivated in different parts of the continents and the content of CN is an important factor for evaluation of its quality and also for fixing the price [5]. Variation of CN contents were observed in the plants grown under different environmental conditions [10] and the rhizomes used for the preparation of herbal products were showing variation in pharmacological activities [11]. For successful usage of turmeric rhizomes and herbal products, a simple, convenient, reliable and cost effective analytical method is required.

Several analytical methods are being used for the quantitative analysis of CMNs which include spectrophotometry [12,13] high performance thin layer chromatography (HPTLC) [14,15] and high performance liquid chromatography (HPLC) [16–18]. Spectrophotometric methods are found to be useful for the estimation of total

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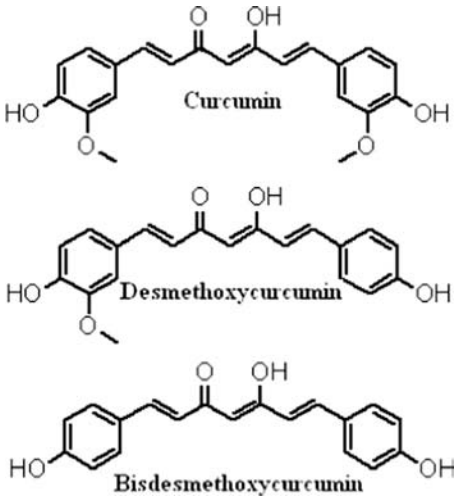

Fig. 1. Chemical structure of the three major curcuminoids (CMNs) in turmeric rhizomes.

content of CMNs, but the composition of individual constituent present in rhizomes cannot be determined.

HPLC is the one of the most widely used technique for separation of CMNs and the degree of separation mostly depends on type of column and eluent used. Capillary electrophoresis (CE) is a viable alternative to HPLC for the analysis of CMNs and often, CE offers a more flexible method development than HPLC [19]. As compared to HPLC, CE does not require any expensive chromatographic column for separation. In addition to that it requires only low volume of electrolytes/buffers for the separation.

CE is currently gaining importance and popularity for the analysis of herbal products [20–22]. This is mainly due to wide utility of fused silica capillary for the separation of compounds both in aqueous [23–26] and non-aqueous medium [27–29] with wide pH range. Low operational cost, easy to maintain, high resolution, and eco-friendly solvent system are additional advantages of this technique. Although, CE methods have been widely used for the analysis of pharmaceutical and herbal products, its application for the determination of CMNs in herbal products is very limited [30,31]. Literature survey also revealed that no method is available for the determination of CMNs in skin cream product. Lechtenberg et al. [30] reported a capillary zone electrophoresis (CZE) method for determination of CMNs, which requires cyclodextrin, a chiral additive to achieve the separation, however, the resolution is low ($\sim$ Rs <1.0). For hydrophobic compound, MEEKC and NACE methods are found to be suitable for better separation. Nhujak et al. [31] developed a microemulsion electrokinetic chromatography (MEEKC) method for the determination CMNs using 2, 4-dinitrophenyl phthalimide as internal standard (IS), which is not commercially available. Using a high concentration of 180 mM SDS which resulted in high back ground noise. In MEEKC, the separation and reproducibility are also often sensitive to the composition of microemulsion, temperature and condition of the capillary wall surface [32]. Hansen and Sheibah [33] compared CZE, MEKC and MEEKC methods for the determination of hydrophobic compounds and found that NACE is the best among them due to good selectivity.

Non-aqueous capillary electrophoresis (NACE) proved to be suitable for the lipophilic compounds [27–29]. Use of non-aqueous background electrolytes in CE for the separation hydrophobic compounds has several advantages such as better solubility and selectivity, lower adsorption of electrolytes and analytes on the capillary wall. Low current generation and ESI-MS compatibility are additional advantages of NACE.

The aim of the present work was to develop a simple, fast and reliable NACE method for the determination of CMNs in turmeric rhizomes products. To test the suitability of the method, the results obtained by the proposed method were compared with the existing spectrophotometric and HPLC methods. To the best of our knowledge, this is the first report for the determination of CMNs by the NACE method.

2. Materials and methods

2.1. Chemicals

All chemicals were of analytical grade and were used without further purification. Sodium tetraborate, hydroxylpropyl-β-cyclodextrin, ammonium acetate, tris(hydroxymethyl) aminomethane, curcumin, and sodium dodecylsulphate (SDS) were purchased from Sigma-Aldrich, Bangalore, India. Methanol, 1-propanol and acetonitrile were procured from SD Fine Chemicals, Mumbai, India. *C. longa* (turmeric) rhizomes were collected from different parts of India. Since, the marker compounds of BN and DN are not available commercially; they were isolated and identified as described in the literature [16,34]. Deionized water was obtained from Millipore Milli-QA10 System (Waters, Germany). All pH measurements were made with an ELICO® LI 615 pH meter (India)

2.2. Sample preparation

2.2.1. Preparation of standard solutions

Stock solutions (1.0 mg/ml) of CN, BN, DN and *p*-aminobenzoic acid (IS) were prepared separately in methanol and stored in amber colored bottles at 4 °C. Desired concentrations of working standard solutions of individual or mixtures were freshly prepared by diluting the stock solution with methanol prior to injection.

2.2.2. Preparation of NACE background electrolyte

Sodium tetraborate (STB) 309.0 mg was accurately weighed and transferred into a 100 ml beaker and 4.0 ml of 100 mM sodium hydroxide (NaOH), 30.0 ml methanol, and 20 ml of 1-propanol were added, stirred well and sonicated for 5 min. The resulting solution consisted of 15.0 mM STB and 7.4 mM NaOH in 2:10:15 (v/v/v) of water, 1-propanol, and methanol respectively. The apparent pH* of the solution was 10.8. The solution was transferred in an airtight bottle and stored in refrigerator. Prior to CE analysis, the solution was filtered through 0.45 μm filter and used for separation.

2.2.3. Extraction of curcuminoids

Turmeric powder, herbal capsules and cosmetic cream were weighed (20–30 mg) separately into a 2.0 ml eppendorf microcentrifuge tube. For turmeric power and herbal capsules, 1.0 ml of methanol, and for cream sample, a mixed solvent (0.7 ml of methanol + 0.3 ml of chloroform) were added. The samples in the tubes were thoroughly mixed and shaken with the help of cyclomixer for 30 s × 3 times and ultrasonicated 5 min × 3 times. The shaking and ultrasonication were performed alternatively to ensure complete extraction of CMNs. All the sample tubes were centrifuged for 5 min. 300 μl of the supernatant solution was filtered into a CE sample vial by using 0.22-μm syringe filter, 20 μl of internal standard was added and mixed well. The pictorial scheme of the extraction procedure is shown in Fig. 2.

2.2.4. Analysis

Capillary electrophoresis separations were performed on a Prince Technologies CEC 750 system (The Netherlands) equipped with PDA and 3D DAx software. The uncoated fused silica capillary

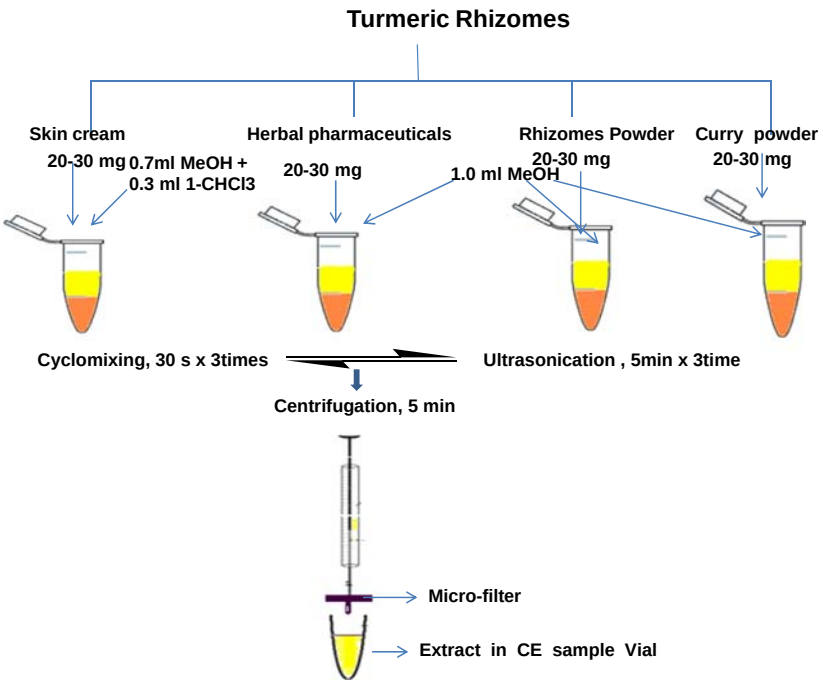

Fig. 2. The pictorial representation of extraction of CMNs in turmeric products.

(Polymicro, Phoenix, AZ, USA) with of 75 μm i.d. and 350 μm o.d., and total length of 50 cm (42.5 cm effective length), was used throughout the analysis.

Before analysis, the capillary was cleaned and conditioned as follows: water (5.0 min), 0.1 M of NaOH (10 min), water (5 min) and finally with BGE for 10 min. For every fifth injection, the capillary was rinsed with a mixture of solution containing methanol: water (3:1, v/v) for 10 min. Sample injection was performed by hydrodynamic injection (25 mbar/12 s). Separation was carried out at reverse polarity by keeping a constant voltage of 28 kV. Detection was performed both at 201 and 496 nm for quantification. Capillary compartment and sample tray temperature were set 25 °C and 20 °C respectively. The BGE vial was replaced after every fifth run, to minimize the chemical contamination and electrolyte depletion. When not in use, the capillary ends were placed in water vial.

For the estimation of total content of CMNs, spectrophotometric method was performed using a Raleigh spectrophotometer (China). Turmeric products were extracted with tetrahydrofuran. The supernatant solution was suitably diluted with methanol and the absorbance was measured at 420 nm [13]. The HPLC instrument used for analyses was an Agilent 1100 series, Agilent Technologies, Inc. The Phenomenex Luna, C18 ($150 \times 4.6\ mm^2$ i.d., 5 μm) column was used for the separation and detection wavelength was set at 425 nm. All other experimental conditions of the instrument were set as described in the literature [17]. The resolution (Rs) and efficiency (N) were calculated using the equations reported [35].

3. Results and discussion

3.1. Method development

The initial trials for the separation of CMNs were performed based on the literature information for separation of phenolic compounds by capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC).

Different concentrations of sodium tetraborate (10–50 mM) at different pH values (8.0–12.0) with or without sodium dodecylsuphate (20–80 mM) and organic modifier (methanol, acetonitrile) were prepared and analyzed. The results of the analyses indicated that none of electrolyte system can separate all CMNs completely. Either partial separation or tailing or peaks broadening were observed under the experimental conditions. Probable reason could be CMNs possess the same q/r ratio under the given electrophoretic conditions.

In capillary electrophoresis, compounds can be separated, if they possess different effective mobilities (μ_{eff}). The μ_{eff} is proportional to its charge (q) and inversely proportional to its salvation (r). Preparations of BGEs with organic solvent (s) often influence the charge or size of the analytes and also enhance the ion-pairing effect [36].

3.2. Influence of BGE composition

The effect of NaOH concentration (0–7.4 mM) on the separation of CMNs was investigated. In NACE, the separation is generally carried out without knowledge of the exact pH value of BGE, because the organic solvent shifts pKa to a larger value with respect to water. CMNs contain two phenolic OH and one enolic ionizable group. In order to ionize CMNs, basic buffer is necessary [30]. The electrophoretic mobility of individual CMNs depends on the degree of ionization and the extent of interaction of analyte with electrophoretic medium. Effect of NaOH concentration on separation is shown in Fig. 3. As the concentration of NaOH is increased, migration time of CMNs decreased and the separation improved significantly. Better separation was shown at 7.4 mM NaOH and therefore, further studies this concentration was chosen.

Different electrolytes (Tris, ammonium acetate and STB) and the solvents viz., methanol, 1-propanol and water were tested for the separation of CMNs. Typical electropherograms obtained with different electrophoretic conditions are shown in Fig. 4. Bad separation and peak broadening were observed with tris and ammonium acetate (Fig. 4(a)–(e)), while, STB (15 mM) provided good baseline separation (Fig. 4(f)). Boric acid [$B(OH)_3$] and

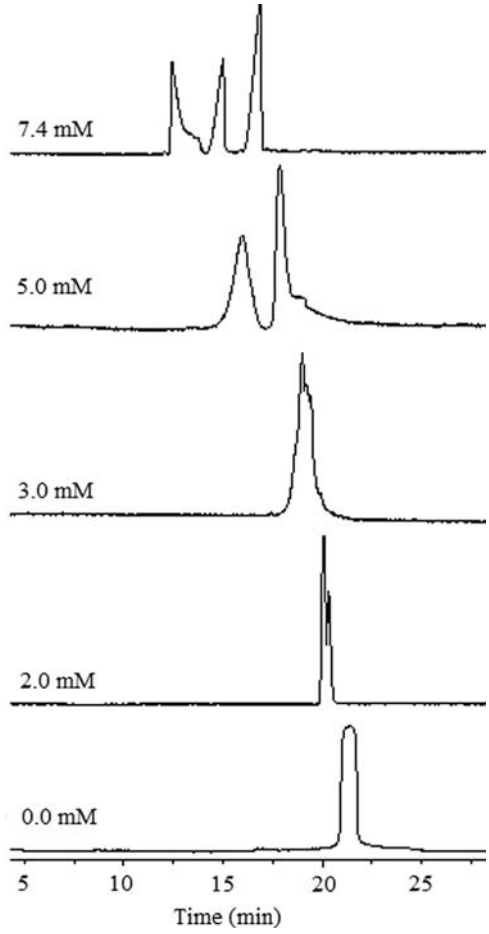

Fig. 3. Typical electropherograms show the effect of sodium hydroxide concentration on the separation of CMNs.

tetrahedral borate [$B(OH)^{4-}$] are known to form a complex with diol, polyhydroxy compounds [37,38] and CMNs [39]. The complex induces change in charge-to-mass ratios of the ligand and this principle is used in the capillary electrophoresis separation of polyhydroxy compounds [38]. The reason for better separation of CMNs on using STB in electrolyte is probably due to the induction of changes in the charge-to-mass ratios of borate-CMNs complex.

The effect of solvent(*s*) on separation was also investigated. It is well known that the organic solvent can alter the electrophoretic behavior of the hydrophobic compounds. Methanol, 1-propanol, and water were tested as solvents. The concentration of NaOH (7.4 mM) and STB (15 mM) were kept constant; different v/v% of water between 5.55% and 9.25% v/v in BGE was varied to evaluate its effect on separation of CMNs. Among the tested level of water, 7.4% v/v provided better separation than other conditions. However, the content of water level $7.4 \pm 1.0\%$ v/v in BGE is not significantly affecting the separation. STB and NaOH are not completely miscible with methanol and 1-propanol, and the use of water ($7.4 \pm 1.0\%$v/v) makes the electrolytes miscible completely. Water not only enhances the solubility of the electrolytes, but also improved the peak efficiency and resolution. After several trials, it was observed that a mixture of H_2O–MeOH–1-propanol (2:15:10, v/v/v) was the best choice of solvent system for separation of the compounds under investigation.

Studies indicated that the addition of SDS [40] and cyclodextrins [41] in NACE improves the separation and selectivity of structurally related compounds. With this in view, the effect of hydroxylpropyl-β-cyclodextrin (HPCD) and SDS surfactant on separation of CMNs were tested. The electropherograms, (a) 20 mM HPCD and (b) 30 mM SDS recorded under those conditions are shown in Fig. 5. The results indicated that there was no improvement on the separation, peak efficiency and precision in migration time on addition of SDS and HPCD to BGE.

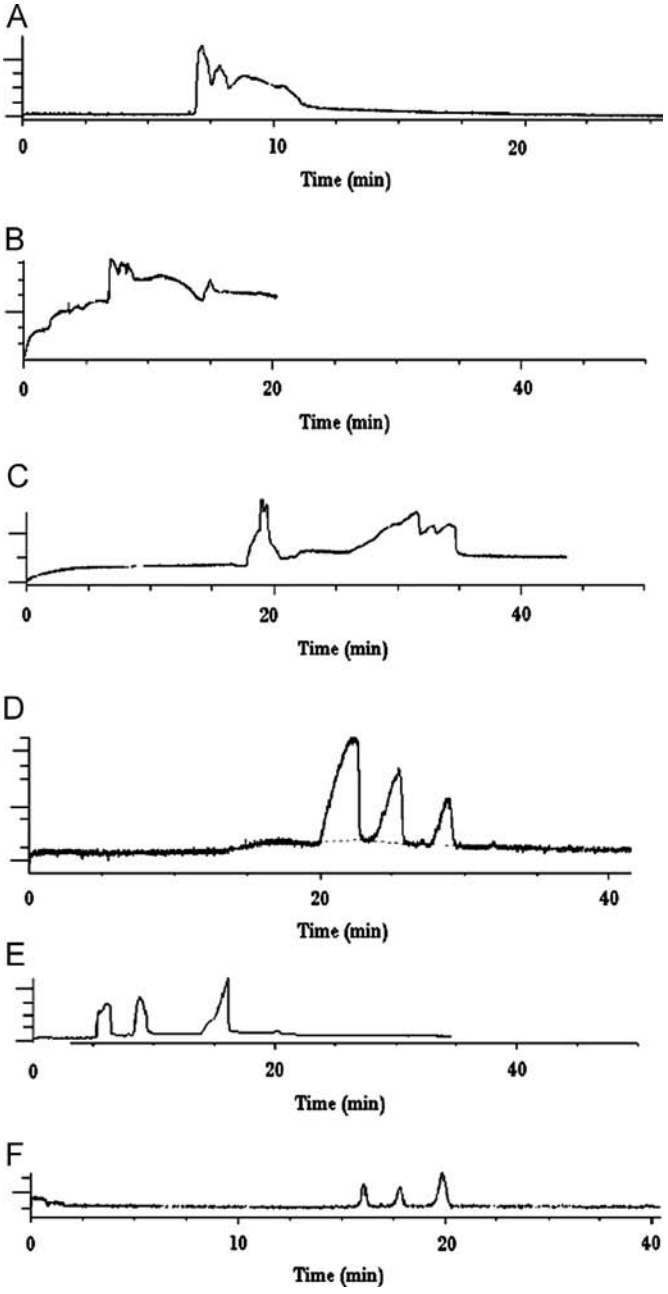

Fig. 4. Electropherograms showing the separations of CMNs in different electrophoretic mediums. (a) 7.4 mM NaOH in H_2O-1-PrOH–MeOH (2:10:15, v/v/v); (b) 20 mM Tris, 7.4 mM NaOH in H_2O–1-PrOH–MeOH (2:10:15, v/v/v); (c) 20 mM ammonium acetate, 7.4 mM NaOH in H_2O–1-PrOH–MeOH (2:10:15, v/v/v); (d) 15 mM STB, 7.4 mM NaOH in MeOH; (e) 15 mM STB, 7.4 mM NaOH in MeOH-1-PrOH (2:1, v/v), (f) 15 mM STB, 7.4 mM NaOH in H_2O–1-PrOH–MeOH (2:10:15, v/v/v. Separation condition: fused silica capillary of 75 μM i.d, effective length of 62.8 cm, separation voltage of 20 kV (reverse polarity).

3.3. Optimization of instrumental parameters

3.3.1. Voltage and injection

While using positive voltage for separation, it was observed that analytes eluted after longer time and therefore, reverse polarity was applied [42] to reduce migration time. Peak efficiency

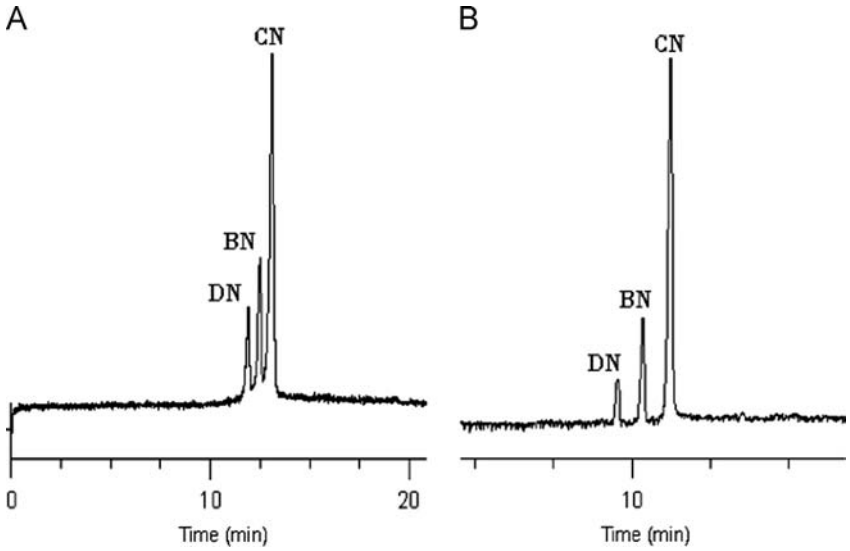

Fig. 5. Effect of (a) 20 mM hydroxylpropyl-β-cyclodextrin (HPCD) and (b) 30 mM sodium dodecyl sulfate in15 mM STB, 7.4 mM NaOH in H_2O–1-PrOH–MeOH (2:10:15, v/v/v) (SDS) on separation of CMNs. Separation condition: fused silica capillary of 75 μM i.d., effective length of 37.8 cm, separation voltage of 28 kV (reverse polarity).

and resolution increased by increasing the voltage from 10 to 28 kV and analysis time is reduced. However, above 28 kV, breakdown of current and higher % RSD value on migration time were observed due to Joule heating. To achieve faster separation and better reproducible migration time, separation voltage of 28 kV was applied. Under the applied separation voltage, a current of 24 μA was observed. In order to increase the sensitivity of detection without affecting the peak shape and resolution of CMNs, injection pressure (10–50 mbar) and duration of injection time (3–18 s) were varied to get optimum injection condition. It was performed by keeping one parameter constant at a time, while varying the other. The injection pressure/time longer than 50 mbar/12 s is caused peak efficiency loss greater than 6% and low peak area precision (%RSD > 4.6, $n=6$). A relatively low injection error was observed at 25 mbar/12 s and therefore, it was optimized.

3.3.2. *Internal standard and electropherograms*

In CE, the use of internal standard (IS) is preferable in order to compensate the injection errors, evaporation loss of solvent and fluctuation due to migration. In previous CZE [30] 3,4-dimethoxy-*trans*-cinnamic acid and MEEKC [31] studies, 2,4-dinitrophenyl phthalimide were used as IS and the detection wavelengths were set at 258 and 214 nm respectively. In NACE system, 3, 4-dimethoxy-*trans*-cinnamic acid is found be unsuitable due to co-migration with BN and also 2, 4-dinitrophenyl phthalimide is not commercially available. Therefore, salicylic acid, p-hydroxybenzoic acid, nicotinic acid and p-aminobenzoic acid were screened to select a suitable IS. Among them p-aminobenzoic acid was found to be suitable and therefore it was used as IS. The other compounds either overlapped with the CMNs or migrated after a longer period of time. Fig. 6 shows the separation of CMNs under optimized condition. Typical electropherogram recorded at 496 nm and in sito UV–visible spectra (inserted); two dimensional contour plot and the electropherogram recorded at 201 nm are shown in Fig. 6(a)–(c) respectively. From the figures it can be seen that all the analytes are well separated and eluted before 12 min. Tailing factors (T) for all the analytes were < 1.03 and demonstrating symmetry of peaks ($T < 2$). Two dimensional contour plot demonstrates the visual appearance of CMNs separation. The CMNs have absorbance both in UV as well as visible region and the absorption maximum for CN, BN and DN were 520, 515 and 496 nm respectively. But internal standard has absorbance only in UV region. Determinations of CMNs in herbal products were performed at 490 and 201 nm for external and internal standard calibration methods respectively.

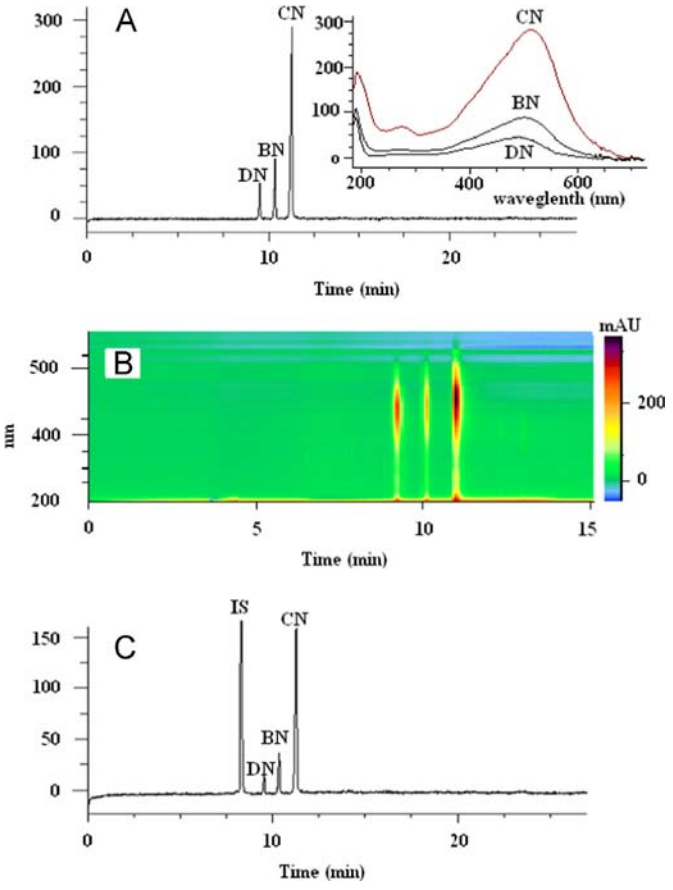

Fig. 6. Electreopherograms showing the separation of CMNs under the optimized NACE electrophoretic conditions. BGE: 15 mM STB, 7.4 mM NaOH in H_2O–1-PrOH–MeOH (2:10:15, v/v/v) (SDS) on separation of CMNs. Separation condition: fused silica capillary of 75 μM i.d., effective length of 42.8 cm, separation voltage of 28 kV (reverse polarity). (a) recorded at 496 nm and the UV spectrum of CMNs (inserted), (b) Two dimensional contour plot of CMNs. (c) Electropherogram of CMNs with IS recorded at 201 nm.

3.4. Extraction of CMNs

To select a suitable solvent for the extraction of CMNs, extractions were performed with a single plant rhizome powder, as described in materials and method Section 2.2.3. Chloroform, methanol, ethanol and 1-propanol were used for extraction. Averages of three replicate analyses were taken and the results are presented in Table 1. Methanol was found to be the most appropriate solvent for extraction of CMNs and results obtained also agreed well with previously reported HPLC method [18]. However, the extraction procedure developed is simple, fast, effective and after filtration the sample can be analyzed without any pretreatments.

3.5. Method validation

The analytical characteristics of the developed method were evaluated in terms of linearity, limits of detection (LOD), quantification (LOQ), precision, accuracy, stability and robustness.

3.5.1. Linearity, LOD and LOQ

Linearity was evaluated by plotting, (a) the corrected peak area (peak area/migration time) versus concentration of the analyte for calibration of external standard method and (b) peak area ratio (peak of analyte/peak area of IS) versus the concentration of the analyte for calibration of internal standard. CMNs absorb at a wavelength of 496 nm, but IS did not have any absorption at 496 nm. However, both CMNs and IS have sufficient UV absorption at 201 nm. Therefore, both the external and internal calibration methods were compared for the determination of CMNs. LOD and LOQ were determined based on signal-to-noise (S/N) ratio of three and ten times respectively. The data obtained are given in Table 2. The data reveals that the proposed NACE method showed good linearity ($R^2 > 0.998$) over the tested concentration and the LOD and LOQ were lower at 496 nm than 201 nm, as the analytes have maximum absorbance at 496 nm.

Table 1
Effect of solvents on the extraction efficiency of CMNs in turmeric rhizome.

Extraction solvent	CN	Content of individual and total CMNs (%) ($n=3$)					
		RSD% ($n=3$)	DN	RSD% ($n=3$)	BN	RSD% ($n=3$)	% Total CMNs
Chloroform	1.81	1.62	0.98	1.64	0.80	1.78	3.59
Methanol	2.05	1.53	1.21	1.62	0.91	1.54	4.17
Ethanol	1.96	1.65	1.16	2.08	0.89	1.72	4.01
1-Propanol	1.89	1.82	1.22	2.40	0.81	1.75	3.92

3.5.2. Precision

Standard solutions of CMNs at three different concentrations (low, medium and high) viz., 20.4, 81.6 and 183.6 μg/ml for BN, 20.8, 83.2 and 187.2 μg/ml for DN, and 20.1, 80.2 and 180.9 μg/ml for CN were analyzed six times in the same day to evaluate the intra-day (repeatability) precision. The solutions were also examined in three consecutive days for inter-day precision. The RSD values for intra and inter-day were lower than or equal to 2.14%.

3.5.3. Accuracy

Accuracy of the methods was evaluated by means of recovery studies. To the previously analyzed and the content of individual curcuminoid known sample i.e. BN (32.4 μg/ml), DN (39.2 μg/ml) and CN (72.2 μg/ml), a known amount of individual CMN standard at two different levels (20.0 and 40.0 μg/ml) were added and analyzed. The mean recoveries for BN, DN and CN ranged between 97.2% and 98.8%, 98.5% and 98.2%, and 97.9% and 97.9% and 98.7% respectively. The RSD values were lower than or equal to 2.62%.

3.5.4. Stability

NACE background electrolyte stored in an airtight amber colored bottle was stable for a week at room temperature (25 °C) and one month in refrigerator (4 °C). The stability of CMNs in the optimized NACE background electrolyte solution was tested by measuring the UV–visible absorption (200–700 nm). The spectra were collected for over 40 min using a Raleigh spectrophotometer at 5 min time interval. The results indicated that the absorbance of CMNs did not change upto 40 min and the probable reason could be that the hydrophobic microenvironment prevents degradation of CMNs. Thus, BGE significantly enhanced the stability of the CMNs.

3.5.5. Robustness

Robustness was checked by making minor changes (± 0.5%) in the optimized experimental conditions, which included composition of BGE, capillary length, temperature and separation voltage. The results of analysis ($n=3$) on peak efficiency, migration shift and peak area were calculated with respect to the optimized conditions. The percent RSD value on peak efficiency, migration shift and corrected peak area were within 1.2, 2.8 and 2.6 respectively, which indicated that the developed method is robust.

3.6. Analytical applications to herbal products

The developed NACE method was applied for the determination of CMNs in herbal products. The electropherogram of herbal products separation is shown in Fig. 7. Representative electropherograms of herbal curcumin capsule (declared content of CN 500 mg), turmeric extract capsule, skin cream, and turmeric

Table 2
Linearity, LOD and LOQ.

Analyte	Tested concentration range (μg/ml)	Linear equation			LOD (μg/ml)	LOQ (μg/ml)
		Slope	Intercept	Correlation coefficient (R^2)		
At 496 nm						
BN	20.4–183.6	0.014335	−0.02131	0.9990	4.9	14.1
DN	20.8–187.2	0.014016	−0.00053	0.9988	5.0	14.6
CN	20.1–180.9	0.013660	0.03302	0.9994	4.2	12.4
At 201 nm with IS						
BN	20.4–183.6	0.004121	−0.001497	0.9992	7.1	19.2
DN	20.8–187.2	0.004656	−0.009871	0.9986	7.2	19.6
CN	40.2–241.2	0.004442	−0.004176	0.9994	8.2	21.8

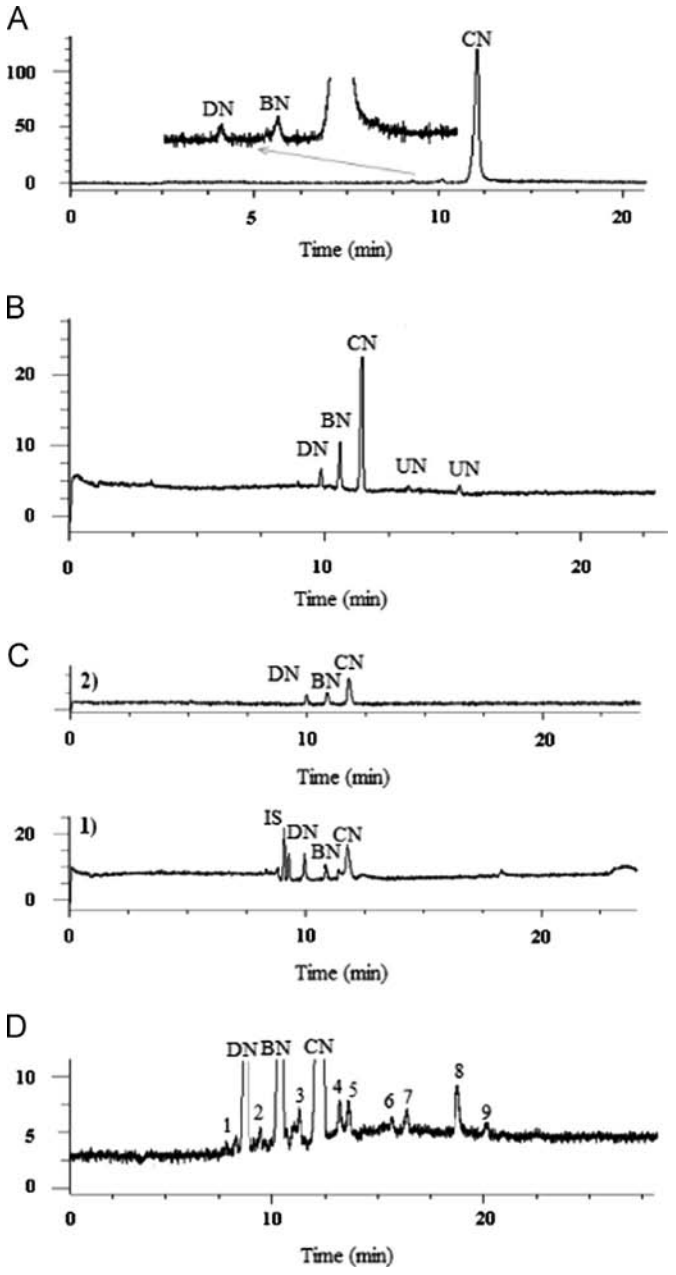

Fig. 7. Analyses of turmeric herbal products under optimized electrophoretic conditions for determination of (a) curcumin capsule, at 496 nm (b) herbal pharmaceuticals, at 496 nm (c) skin cream (1) scanned at 201 nm to show interferences, (2) scanned at 496 nm, (d) To show the resolution CMNs and related compounds at 427 nm of turmeric extract. For separation conditions refer Fig. 6.

rhizomes are depicted in Fig. 7(a)–(d) respectively. The peaks were identified and confirmed by comparing with migration time and absorbance spectrum with the corresponding standard. Individual and total CMNs percentage was calculated by using both external (496 nm) and internal (201 nm) standard methods. The total CMNs content was calculated based on the sum of the BN, DN and CN. There is no significant difference in the content estimated by external and internal standard methods. The percentage of CN and CMNs in herbal pharmaceuticals determined by this method was also compared with the spectrophotometric method (SPM). The total content of CMNs estimated by SPM (427 nm) was found be higher than that estimated by the NACE method. Probable reason could be that the SPM is not selective to the target CMNs alone, and there are several other minor constituents having absorption at 427 nm also contribute to this. Variation of individual and total content of CMNs was observed. The reason could be that the raw materials obtained were from different sources [5]. The proposed NACE method is also suitable for the detection and determination trace levels of BN, DN in CN.

CMNs extract is also used in skin cream (15% of turmeric extract in non-greasy base) preparation for the treatment of wounds, ulcers, boils, blisters and acne. After analyzing the extract at 201 nm (Fig. 7(c1)), it was observed that some matrix component peak overlapped with analyte peak. However, the interference due to matrix component was not observed at wavelength 496 nm (Fig. 7(c2)). Further, the detection wavelength at 496 nm provides lower background noise, high sensitivity and low interference to other compounds. Total content CMNs estimated in skin cream extract by the NACE and SPM methods were 95.3 and 90.6% respectively. Lower content estimated by SPM is due to the interference of cream base excipients. Fig. 7(d) shows the turmeric rhizomes extract analyzed at 427 nm by NACE. Several minor constituents other than major CMNs were observed from the electropherogram, which showed the resolving power of the developed method for separation of related compounds.

3.6.1. *Comparison of NACE with HPLC*

The developed method was also compared with HPLC method [17] for the determination of a few selected rhizomes and herbal products There were no significant differences in content (%) calculated by both methods (Table 3).

Under optimized NACE separation conditions, the tailing factor, efficiency, resolution, LOD and LOQ of CMNs were calculated and compared with the values reported in CZE, MEEKC and HPLC methods published in recent years. NACE showed better resolution and peak efficiency than other methods. The data are summarized in Table 4. As far as sensitivity is concerned; NACE is less sensitive than the HPLC. The reason for lower sensitivity of NACE method was mainly due to narrow path length of the capillary and low

Table 3
Comparison of NACE and HPLC methods for the content (%) CMNs.

Sample	Content of individual CMNs (%)											
	M-NACE ($n=3$)						HPLC ($n=3$)					
	BN	RSD%	DN	RSD%	CN	RSD%	BN	RSD%	DN	RSD%	CN	RSD%
TR	1.18	2.02	1.54	1.73	2.71	1.82	1.16	1.21	1.54	1.11	2.73	1.71
SP	1.13	1.67	0.89	2.10	1.65	1.66	1.14	1.32	0.91	1.42	1.66	1.58
HP	0.54	1.82	0.76	1.92	1.52	1.54	0.53	1.78	0.77	1.33	1.54	1.51

TR=turmeric rhizomes (Salem), SP=curry powder, HP=herbal pharmaceutical.

Table 4
Comparison of the NACE with CE and HPLC for the peak profiles and limits of detection and quantification.

Data	Proposed NACE			CZE (Ref. 30)			MEEKC (Ref. 31)			HPLC (Ref. 17)		
	BN	DN	CN	BN	DN	CN	BN	DN	CN	BN	DN	CN
T	1.02	1.02	1.03	N/A			N/A			1.03	1.06	1.06
N	106,520	88,027	86,562	N/A			N/A			61,109	56,768	56,860
Rs	4.92		3.84	~1.0			~2.4		~2.4	2.66		2.72
LOD	4.9	5.0	4.2	10.0			5.5	4.7	5.7	0.08	0.84	0.90
LOQ	14.1	14.6	12.4	NA			13.3	10.6	10.7	0.23	2.53	2.73

T=tailing factor, *N*=efficiency, Rs=resolution, LOD and LOQ=μg/ml, N/A=not available.

volume of sample injection. However, high resolving power, low adsorption of analyte and buffer on capillary wall are the advantages of this method.

4. Conclusions

This is the first report for the simultaneous determination of CMNs using non-aqueous capillary electrophoresis. Most commonly and commercially available chemicals were used for the preparation of BGE and internal standard. The resolution was better than the existing CE and HPLC methods. The extraction method proposed in this work is simple, fast and effective. Around ten samples can be simultaneously extracted under identical conditions. Therefore, the overall extraction time and the error due to sample preparation are significantly reduced. The method is most suitable for fast screening of turmeric rhizomes and is cost-effective. Further, the method is also suitable for the determination of trace levels of BN, DN in CN. Capillary blockage had never occurred during the analysis (~5 months) of turmeric samples using non-aqueous background electrolyte and it is an additional advantage of this method.

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