DEVELOPMENT AND IMPLICATION OF A CAPILLARY ELECTROPHORESIS METHODOLOGY FOR CIPROFLOXACIN, PARACETAMOL AND DICLOFENAC SODIUM IN PHARMACEUTICAL FORMULATIONS AND SIMULTANEOUSLY IN HUMAN URINE SAMPLES

AMBER REHANA SOLANGI[1*], SAIMA QAYYUM MEMON[2], ARFANA MALLAH[3], NAJMA MEMON[1], MUHAMMAD YAR KHUHAWAR[2] AND MUHAMMAD IQBAL BHANGER[1]

[1]*National Center of Excellence in Analytical Chemistry, University of Sindh, Jamshoro, Pakistan*
[2]*Institute of Advance Research Studies in Chemical Sciences, University of Sindh, Jamshoro, Pakistan*
[3]*MA Kazi Institute of Chemistry, University of Sindh, Jamshoro, Pakistan*

ABSTRACT

This work studies the development of a simple and fairly rapid methodology for simultaneous determination/ separation of three frequently co-administered drugs; ciprofloxacin (CIP), paracetamol (PCT) and diclofenac sodium (DIC) using capillary electrophoresis (CE) with UV detection at 260 nm. Separation was achieved in only 6.5 min with a simple buffer of sodium tetraborate (50 mM) at pH 9.0. The Parameters affecting the separation and detection were optimized. The calibration curves were linear in the range of 5-500 µg/mL for CIP, 5-250 µg/mL for PCT and 1-125 µg/mL for DIC sodium under the optimized conditions. The lower limit of detection (LOD) was found to be 1 µg/mL for CIP & PCT and 0.5 µg/mL for DIC. The method was successfully used for the analysis of drugs in commercial pharmaceutical formulations and simultaneously from patient's urine sample with RSD 0.5-2.4%. Results obtained with CE method are compared with standard HPLC procedure and were found in good agreement.

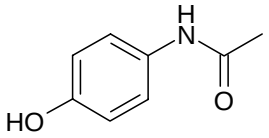
Keywords: Capillary electrophoresis, quinolone, antibiotics, ciprofloxacin, paracetamol and diclofenac sodium.

INTRODUCTION

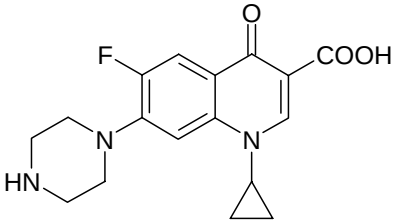
Ciprofloxacin (CIP) is a broad spectrum antibiotic belonging to floroquinolones (FQs). The FQs are a common choice of antibiotics used in many human and animal applications worldwide and the CIP in its hydrochloride form is perhaps the most popular FQ. Its importance has been increased due to bacteriological (anthrax) terrorists' attack threats because it is commonly used in treatment of *Bacillus anthracis* infection (Michalska *et al*., 2004). The CIP has also been very popular lately in the USA because it is used as an approved drug for the treatment of the inhaled form of anthrax (Swartz, 2001). Likewise, it is also a very commonly prescribed antibiotic in Pakistan.

Bacterial infection always results fever and painful inflammation in humans. Therefore, co-administration of antibiotics with analgesics and anti-inflammatory drugs is very common to avoid these effects. Paracetamol (PCT) is used as an analgesic and antipyretic drug to relieve fever, headaches, and other minor aches and pains while diclofenac (DIC) is a non-steroidal anti-inflammatory drug (NSAID) taken to relieve inflammation and severe pain. Fig. 1 shows the chemical structures of all three drugs.

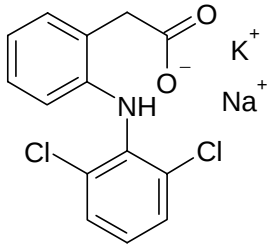
When drugs are administered in different combinations, the routine analysis should apply only one method to analyze all the active ingredients in the biological fluids, such as urine or blood serum, just to reduce errors and the determination time per sample. Therefore, it is important to develop an easy and rapid method to analyze different drugs in a single run with shortest analysis time.



1. Paracetamol



2. Ciprofloxacin



3. Diclofenac sodium

Fig. 1: Chemical structure of drugs.

**Corresponding author:* e-mail: ambersolangi@gmail.com

No reports have been found in the literature for CIP, PCT and DIC being simultaneously determined. However, several methods are reported about the separation and determination of different antibiotics, analgesics and anti-inflammatory drugs. Separation of the drugs is conventionally carried out using liquid chromatography (Conkle *at el*., 2009, Smet *et al*., 2009, Witte *et al*., 2007, Aksoy *et al*., 2007, Espinosa *et al*., 2006, Satinsky *et al*., 2004, çenyuva and Özden, 2002). However, successful assay procedures have also been reported using capillary electrophoresis (CE) (Guzman *et al*., 2008, Macia *et al*., 2007, Michalsk *et al*., 2004, Jin and Zhang, 2000). CE is advantageous over HPLC in terms of solvent consumption, small sample volumes and shorter analysis time (Vera-Candioti *et al*., 2010, Ko. *et al*., 2009, Solangi *et al*., 2009, Solangi *et al*., 2010). Current study is aimed at developing a simple and fairly fast separation and determination procedure for CIP, PCT and DIC sodium in pharmaceutical formulations and simultaneously in human urine samples using capillary electrophoresis.

MATERIALS AND METHODS

Instrumentation

The CE instrument used was Beckman Coulter P/ACE MDQ (Beckman Instruments Inc. Fullerton, CA) attached with auto sampler, photo-diode array detector and a data handling system comprising an IBM PC and P/ACE system MDQ (32 Karat) software. The fused silica capillaries with dimensions: 57 cm total length, 50 cm effective length, 75 µm ID, 375 µm OD obtained from Beckman. The temperature of the capillary and the sample was maintained at 25°C. The capillary was regenerated and conditioned everyday with methanol for 1 min, followed by water for half min, hydrochloric acid (0.1 M) for 2 min, water for half min, sodium hydroxide (0.1 M) for 2 min, water for half min and then running electrolyte for 2 min. The pH measurement was made with Orion 420A pH meter.

Reagents and solutions

All reagents were of analytical grade and solvents were of chromatography purity. Pure drug standard compounds of CIP, PCT and DIC were obtained from Abbott Laboratory, (Pvt.) Ltd. Karachi, Pakistan. Methanol, sodium tetraborate, sodium hydroxide and boric acid of GR grade obtained from E-Merck, Germany. The pH of sodium teteraborate was adjusted from 5 to 10 with boric acid or sodium hydroxide and concentration was adjusted to constant value of 50 mM. Buffer electrolyte solutions were prepared fresh daily. All the solutions were prepared with deionized water.

Standard solution

Stock solutions of each drug containing 0.1 g of CIP, PCT and DIC prepared by dissolving drug in 100 mL of deionized water and stored at cool place.

CE procedure

An aliquot of solution containing CIP (5- 500 µg/mL), PCT (5- 250 µg/mL), and DIC sodium (1-125 µg/mL), was placed in screw cap vial (1.5 mL). For preconditioning, the capillary was washed with sodium hydroxide (0.1 M) for 2 min., with water for half min and then with the running buffer for 2 min for equilibration. Then the sample was injected by auto sampler by hydrodynamic (injection pressure 0.5 psi and injection time 4 s) method. The capillary was again rinsed with water for 0.5 min. The electropherogram was recorded by using 50 mM sodium tetra borate (pH 9) as a run buffer at an applied voltage 30 kV and detection wavelength 260 nm.

HPLC procedure

HPLC analysis of individual drug was performed as given in British Pharmacopeia 2007. A Hitachi model L-6200 pump with UV-Vis detector model Hitachi L-4200 was employed using reverse phase C18 inertsil ODS-3 (250mm × 4.6 mm) column. For CIP, the selected wavelength was 278 nm and solvent compositions were acetonitrile and phosphoric acid 13:87 with flow rate of 1.5 mL/min; and injection volume 50µL. DIC and PCT were determined at 254 nm and 245 nm respectively with mobile phase composition of phosphate buffer and methanol (44:66). A CSW32 data integration software was used for calibration and quantification.

Pharmaceutical samples

Ten tablets of each; Novidate (Sami Pharmaceuticals (Pvt) Ltd. Karachi, Pakistan) for the analysis of CIP, Panadol and Paracetamol (Glaxo Smith Kline Pakistan Ltd.) for PCT and Neurofenac (Novartis, Jamshoro, Pakistan) for DIC sodium were grinded finely to powder and about 5 to 10 mg for each active drug was diluted in water. The final volume was adjusted to 10 mL. The solution was shaken thoroughly to dissolve, and sonicated for 15 min. The final solution was filtered through filter paper (Whatman #42) and electrophoretic procedure was followed for a neat solution.

Urine Samples

The urine sample was collected from a volunteer about 8 hrs after administration of tablets Novidate (250 mg CIP), Panadol (500 mg PCT) and Neurofenac (50 mg DIC). The sample was filtered through filter paper (Whatman #42) and 1 mL was diluted to 10 mL. The clean solution was run for recording electropherogram following electrophoretic procedure.

Recovery analysis

Urine sample (1 mL) was added (50 µg) of drug and volume was adjusted to 10 mL. The analysis was carried out using electrophoretic procedure. The quantitation was carried out form increase in response from calibration curve.

RESULTS

Optimization of CE parameters

Selection of Buffer type, pH and concentration

In order to obtain the optimum CE conditions, different buffers types like sodium hydrogen phosphate, sodium phosphate borate and sodium tetraborate in different pH range were investigated. Among all, sodium tetraborate has showed best results in terms of peak shape, resolution factor (>1.5), selectivity and sensitivity. Therefore it was optimized and selected. The concentration of buffer is also a critical parameter to optimize. Four concentrations of sodium tetraborate (25-100 mM) were studied under similer instrumentation conditions (voltage, injection time, temperature, wavelength, etc.). A maximum voltage (+30 kV) was used to minimize current disruption. The optimal separation was observed with 50 mM concentration.

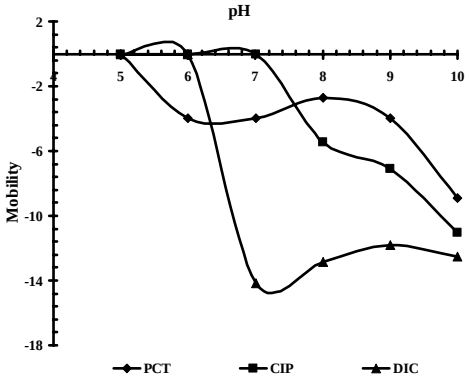


Fig. 2: Plot of pH vs. electrophoretic mobility.

Effects of applied potential and Injection Time

The applied voltage was optimized by studying the voltage within 20-30 kV at an interval of 5 kV. An increase in migration time has been observed by decreasing voltage, with no betterment in the resolution of drugs. Sample injection time (2-10 s) and pressure (0.1-1 psi) were also examined to get a lower detection limit with better quality of peak shape and reproducibility. An injection pressure of 0.5 psi and duration of 4 s injection time showed better results and was selected as optimized injection time and pressure for sample injection.

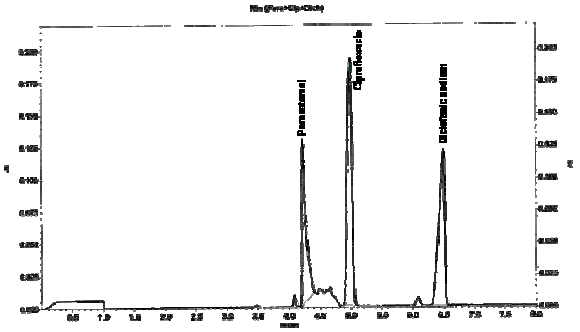


Fig. 3: Typical Electropherogram of mixture of PCT, CIP and DIC sodium: CE conditions: 30 kV, 260 nm; buffer: (50 mM) Sodium tetraborate pH-9.

DISCUSSIONS

The effect of pH of the buffer electrolyte has been optimized by plotting pH verses electrophoretic mobility. The calculation of electro-phoretic mobility for each of the drug was done (Table 1) using equation previously used (Solangi *et al*., 2009). Fig. 2 clearly shows that separation of all drugs was not possible in pH lower than 6.0 since a broad peak for all the drugs at same place has been observed. Therefore, the pH interval within 6.0-10.0 was studied to explore the migration trend of the standard mixture of CIP, PCT and DIC sodium. At pH 6, only two peaks appeared one for PCT and one for CIP and DIC sodium, two of them migrated with same migration time. By increasing the pH to 7 and 8, all three peaks appeared and separated with poor peak shape. Whereas at pH 9, separation was very good with better peak shape while at pH 10 again poor peak shape and less sensitivity was observed. Consequently, pH 9 buffer was optimized for separation of all three drugs. Fig. 3 shows the separation of all three drugs at optimized conditions.

On the basis of results obtained the applied voltage of +30 kV was selected to achieve the shortest analysis time and the highest separation efficiency.

VALIDATION OF THE METHOD

Linearity

The method linearity was verified in the concentration range of 5-500 µg/mL for CIP, 5-250 µg/mL for PCT and 1-125 µg/mL for DIC sodium, in 03 replicates prepared at 06 concentration levels. The calibration curves plotted for each of drug and response was linear with coefficient of determination (R^2) = 0.997 for PCT, 0.997 for CIP and 0.998 for DIC sodium (table 1). The detection limits measured as the signal to noise ratio of 3:1 were obtained within 0.5 to 1 µg/mL and limit of quantitation (LOQ) measured as three times the detection limit was calculated within 1.65 to 3.3 µg/mL (table 1).

Precision

The intra-assay precision (repeatability) was determined by total analysis of six replicates samples, under the same conditions, by the same analyst, and on the same day. The reproducibility of the separation in terms of migration time and peak area/ height for all three drugs was examined (n=6) and RSD were obtained 0.5-1% and 2.1-4.1% respectively (table 1).

Accuracy

The accuracy of the method was evaluated by spiking a known amount of each drug in urine sample. Recovery was obtained more than 95% for all added drugs (table 3).

Selectivity

The signal in terms of migration time and peak shape of the three drugs in the synthetic mixtures containing the

Table 1: Analytical parameters of determination

S. No.	Name of Drug	Coefficient of Determination R^2	Range (µg/mL)	Migration Time (min)	Mobility μ_{ep} (cm^2 /kVmin (at C.L 95%)	LOD µg/mL	LOQ µg/mL	% RSD (n=6)			
								Peak Area	Migration Time	Intra-day error	Inter-day error
1	Paracetamol	0.997	5-250	4.2	-3.96 ± 0.001	1.0	3.3	4.1	1.0	0.1	0.1
2	Ciprofloxacin	0.997	5-500	5.0	-7.11 ± 0.003	1.0	3.3	2.5	0.54	0.2	0.2
3	Diclofenac sodium	0.998	1-125	6.5	-11.79 ± 0.001	0.5	1.65	2.1	0.51	0.1	0.2

Table 2: Recovery assay from pharmaceutical preparations

S. No.	Name of compound	Name of Tablet	Amount labeled (mg/Tab)	Amount found (mg/ Tab)	%RSD
1	Ciprofloxacin	Novidate	250	225.5	3.0
2	Diclofenac sodium	Neurofenac	50	45.05	2.1
3	Paracetamol	Panadol	500	555	2.5
		Paracetamol	500	550	2.6

Table 3: Recovery Assay from Biological Fluids (Urine)

S. No.	Name of compound	Name of Tablet	Amount Taken (mg/Tab)	Amount added (µg)	Amount found (µg/mL)	%RSD	% Recovery	Amount found by HPLC (µg/mL)
1	Ciprofloxacin	Novidate	250	0.00	144.5	2.1		143.2
				50	48.7	2.0	97.4	50.2
2	Paracetamol	Panadol	500	0.00	15	1.7		15.2
				50	47.3	1.2	94.6	47.1
3	Diclofenac sodium	Neurofenac	50	0.00	N.D*	--	---	N.D*
				50	49.1	1.5	98.2	52

*N.D= Not Detected

analytes and common additives (glucose, lactose, sorbitol, gum arabic, starch, magnesium stearate, methylparaben and propylparaben) was compared with the response of standard drug solutions. The additives were added at least twice the concentration of the drug. In all cases, new very small peaks were observed without peak shape/ migration time alteration in original electropherograms. The additives did not interfere with the determination with relative % error within ±1.5%. It is important to consider that method is fairly selective in the determination of the three active ingredients.

Sample analysis of commercial pharmaceutical preparation

The procedure was examined for the analysis of three drugs from pharmaceutical preparations. A fresh calibration curve was prepared followed by the analysis of the drug from pharmaceutical preparation. The results of analysis for all the drugs in four pharmaceutical preparations agreed with the labeled values with RSD (n=6) within 2.1-3.0% (Table 2).

Sample analysis of human urine

The urine sample of a volunteer was collected after 8 hrs administration of drugs CIP (250mg), PCT (500mg) and DIC sodium (50mg). The sample was analyzed after 10 times dilution with water. The amount of drugs from urine was observed 144.5 µg/mL for CIP with RSD 2.1%, 15 µg/mL of PCT with RSD 1.7% and DIC sodium was not detected (Fig. 4). The possible reason for not detecting DIC is that it dissolves slowly in intestine and can not be excreted in much amount after 8 hrs of administration. Pooled samples should be collected after three times DIC dosages therapy in a day. The recovery from urine was calculated by spiking with 50 µg of each of the drug (Fig. 5) and it was found to be 97.4% for CIP, 94.6% for PCT and 98.2% of DIC sodium (Table 3).

The developed CE procedure was validated by comparing the real sample data with standard assay procedure (British pharmacopeia 2007) for single drug using HPLC. The data obtained is given in Table 3. The value of correlation coefficient (0.99) shows that CE assay procedure is equally good in terms of accuracy.

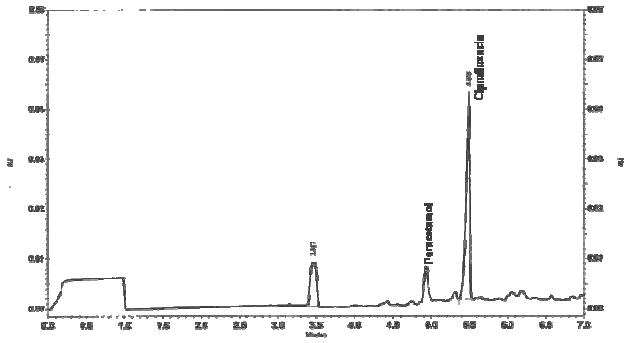


Fig. 4: Typical Electropherogram of Urine Sample of a patient. CE conditions same as Fig. 3.

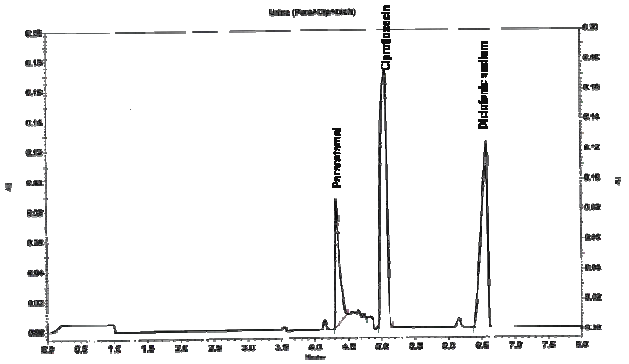


Fig. 5: Typical Electropherogram of Urine Sample added with 50 µg of CIP, PCT and DIC sodium. CE conditions same as Fig. 3

CONCLUSION

The developed methodology is considerably easy and rapid; it determines the three drugs only in 6.5 min in a single run with simple sample preparation. Even urine samples do not need any pretreatment and could be analysed without any interference of the matrix. This method could also be applied to the routine analysis of pharmaceuticals.

REFERENCES

Aksoy B, Kucukguzel I and Rollas S (2007). Development and validation of a stability-indicating HPLC method for determination of ciprofloxacin hydrochloride and its related compounds in film-coated tablets. *Chromatographia*., **66**: S57-S63.

çenyuva H and Özden (2002). Simultaneous High-Performance Liquid Chromatographic determination of paracetamol, phenylephrine HCl, and chlorpheniramine Maleate in pharmaceutical dosage forms. *J. Chromatogr. Sci*., **40**: 97-100.

Conkle, JL, Lattao, CV, White JR and Cook RL (2009). Pharmaceutical analysis for environmental samples: Individual and simultaneous determination of Ciprofloxacin, Ofloxacin and Norfloxacin using an HPLC with fluorescence and UV detection with a wetland soil matrix. *Anal. Lett*., **42**: 2937-2950.

Espinosa-MA, Pena AM de la, Gomez DG and Canada CF (2006). HPLC determination of ciprofloxacin, cloxacillin, and ibuprofen drugs in human urine samples. *J. Sep. Sci*., **29**: 1969-1976.

Guzman NA, Blanc T and Phillip TM (2008). Immunoaffinity capillary electrophoresis as a powerful strategy for the quantification of low-abundance biomarkers, drugs, and metabolites in biological matrices, Electrophoresis, Special Issue: Affinity and Immunoaffinity CE and CEC, **29**: 3259-3278.

Jin W and Zhang J (2000). Determination of diclofenac sodium by capillary zone electrophoresis with electrochemical detection. *J. Chromatogr. A*., **868**: 101-107.

Ko JY, Chang CY, Yang YH and Chen SH (2009). Rapid determination of ciprofloxacin in cerebrospinal fluid by micellar electrokinetic chromatography with direct sample injection and its application in tuberculosis meningitis. *J. Liq. Chromatogr. Rel. Tech*., **32**: 418-431.

Macia A, Borrull F, Calull M and Aguilar C (2007). Capillary electrophoresis for the analysis of non-steroidal anti-inflammatory drugs. *Trends in Anal. Chem*., **26**: 133-153.

Michalsk K, Pajchel G and Tyski S (2004). Determination of ciprofloxacin and its impurities by capillary zone electrophoresis. *J. Chromatogr. A*., **1051**: 267-272.

Michalska K, Pajchel G, Tyski S (2004). Determination of ciprofloxacin and its impurities by capillary zone electrophoresis. *J. Chromatogr. A*., **1051**: 267-272.

Satinsky D, Neto I, Solich P, Sklenaova H, Conceicao M, Montenegro BSM and Araujo AN (2004). Sequential injection chromatographic determination of paracetamol, caffeine, and acetylsalicylic acid in pharmaceutical tablets. *J. Sep. Sci*., **27**: 529-536.

Smet JDe, Boussery K, Colpaert K, Sutter PDe, Paepe PDe, Decruyenaere J and Bocxlaer JV (2009). Pharmacokinetics of fluoroquinolones in critical care patients: A bio-analytical HPLC method for the simultaneous quantification of ofloxacin, ciprofloxacin and moxifloxacin in human plasma. *J. Chromatogr. B*., **877**: 961-967.

Solangi AR, Memon SQ, Khuhawar MY, Bhanger MI and Mallah A (2009). A capillary zone electrophoretic method for simultaneous determination of seven drugs in pharmaceuticals and in human urine. *JAOAC International.* **92**(5): 1382-1389.

Solangi AR, Memon SQ, Khuhawar MY, Bhanger MI, Mallah A (2009). Quantitative separation of oxytocin, norfloxacin and diclofenac sodium in milk samples using capillary electrophoresis. *Biomed. Chromatogr*., **23**: 1007-1013.

Solangi AR, Memon SQ, Mallah A, Memon N, Khuhawar MY and Bhanger MI (2010). Determination of ceftriaxone, ceftizoxime, paracetamol and diclofenac

sodium by capillary zone electrophoresis in pharmaceutical formulations and in human blood serum. *Turk. J. Chem*., **34**: 921-933.

Swartz MN (2001). Recognition and Management of Anthrax – An Update. *N. Engl. J. Med*., **345**: 1621-1626.

Vera-Candioti L, Olivieri AC and Goicoechea HC (2010). Development of a novel strategy for preconcentration of antibiotic residues in milk and their quantitation by capillary electrophoresis. *Talanta*., **82**: 213-221.

Witte BDe, Dewulf J, Demeestere K, Ruyck MDe and Langenhove HV (2007). Critical points in the analysis of ciprofloxacin by high-performance liquid chromatography. *J. Chromatogr. A*., **1140**: 126-130.