Method development for the simultaneous determination of paracetamol and diclofenac in pharmaceutical formulations by capillary zone electrophoresis

Nguyen Thi Huong Giang, Vi Thi Yen Nhi, Nguyen Van Dung, Thai Khoa Bao Chau
Faculty of Pharmacy, Hue University of Medicine and Pharmacy, Hue University

Abstract

Background: Analgesic therapy with the combinations of active ingredients having different mechanisms of action is beneficial for reducing the therapeutic dose and side effects. Therefore, multi-ingredients pharmaceutical preparations such as the combination of paracetamol and diclofenac are becoming more popular on the market. **Objectives:** (1) Developing a capillary zone electrophoresis method for determining simultaneously paracetamol and diclofenac in pharmaceutical formulations (2) Applying this method on the products circulated on the market. Materials and methods: Paracetamol and diclofenac in Zengesic and Ripaigesic film-coated tablets were used in this research. The method was developed and validated according to AOAC 2016 and ICH 2005 guidelines. **Results:** The procedure was developed using the Agilent 7100 CE electrophoresis system with the following electrophoresis conditions: uncoated fused-silica capillary column of a total length of 50 cm (effective length 41.5 cm), sodium tetraborate buffer solution 50 mM (pH = 9), the voltage applied to both capillary ends 30 kV, sample injection mode 35 mbar for 4s, detection with a PDA detector at 276 nm. The method was validated for the capillary zone electrophoresis system compatibility, specificity, linearity range, precision, and accuracy in accordance with AOAC standards. **Conclusions:** The developed capillary zone electrophoresis method can be applied to simultaneously determine paracetamol and diclofenac in pharmaceutical formulations on the market.

Keywords: *Capillary zone electrophoresis, paracetamol, diclofenac.*

1. BACKGROUND

Paracetamol is an antipyretic and analgesic drug with the mechanism of inhibiting prostaglandin synthesis in the central nervous system. However, this active ingredient inhibits both cyclooxygenase-1 and cyclooxygenase-2 poorly, so it has the only limited anti-inflammatory ability. Diclofenac sodium, a salt of diclofenac, is a popular nonsteroidal anti-inflammatory drug that has a strong anti-inflammatory effect due to the inhibition of prostaglandin synthesis in peripheral inflammatory organizations. Some studies have shown that analgesic therapy combining drugs that act on different pain mechanisms may be beneficial in reducing the dose of each component and also reducing side effects. Therefore, nowadays, products combining paracetamol and diclofenac are available on the market to solve the problem of managing mild to moderate pain, which typically has the synergistic analgesia in case of musculoskeletal disease, toothache or postoperative pain [9].

In the world, there have been a number of studies on the simultaneous quantify of paracetamol and diclofenac by different methods, in which the most commonly used methods are high - performance liquid chromatography (HPLC) [7], derivative spectroscopy [10], and capillary electrophoresis (CE) [11].

In Vietnam, paracetamol and diclofenac were simultaneously quantified by high-performance liquid chromatography [3], and derivative spectroscopy [2]. However, up to now, there have been no domestic studies that have announced the simultaneous quantification of these two active ingredients by capillary electrophoresis.

In order to propose a method for simultaneously quantifying the mixture of two components that can be applied in drug quality control, and also contribute to the efficient use of the capillary electrophoresis system with many advantages such as separation efficiency, short analysis times, and saving consumable supplies, we carried out this research to develop a capillary electrophoresis method to simultaneously quantify paracetamol and diclofenac in pharmaceutical formulations.

Corresponding author: Thai Khoa Bao Chau, email: tkbchau@huemed-univ.edu.vn
DOI: 10.34071/jmp.2020.7.2
Received: 6/8/2020, Accepted: 14/9/2020

2. MATERIALS AND METHODS

2.1. Materials

Secondary standard: Paracetamol: content 99%, control number 041519.04 follow Vietnam Pharmacopoeia standards; diclofenac sodium: content 99.51%, control number 0517047.04 of National Institute of Drug Quality Control.

Reagents: Zengesic film-coated Tablets of STADA-VN Joint Venture Co., Ltd (registration number: VD-19193-13, lot number: 540518, expiration date: 26/05/2020) and Ripaingesic film-coated tablets of Thanh Nam pharmaceutical production trading company limited (registration number: VD-19227-13, lot number: 230618, expiration date: 06/2021)

Chemicals: sodium tetraborate (Na2B4O7), sodium hydroxide (NaOH), boric acid (H3BO3), orthophosphoric acid (H3PO4), disodium hydrogen phosphate (Na2HPO4), methanol (MeOH, Merck, Germany), double-distilled deionized water)

Instrumentation – equipment: Agilent 7100 capillary electrophoresis system, HI 2550 - 02 pH meter (Hanna, Italy), double distilled water machine A400D (UK), analytical balance HR-250AZ (Korea), refrigerator preserved sample TOSHIBA (Japan), elmasonic S100H ultrasonic cleaner (Germany), fused – silica capillaries Agilent Technology (USA); precision glassware: volumetric flasks type 10 ml, beaker, micropipette…

2.2. Methods

Preparation of standards

Standard stock solutions: Standard stock solutions of paracetamol and diclofenac were prepared by transferring 10 mg of the drug into two separate 10 ml volumetric flasks having 5 ml of buffers and were ultrasonicated for 5 minutes. Finally, the volume was made up with suitable buffers, which gave 1 mg/ml stock solutions.

Standard mixture solution: The standard mixture solution was prepared by adding 1 ml of paracetamol stock solution and 0.1 ml of diclofenac stock solution in a 10 ml volumetric flask, then added the buffer to the mark and mixed well. The obtained concentration of paracetamol was 100 µg/ml and diclofenac was 10 µg/ml in this standard mixture solution.

Preparation of calibration standards

Suitable aliquots of the above-prepared stock solutions were transferred into a series of 10 ml volumetric flasks, then completed to final volumes with sodium tetraborate 50 mM, pH = 9 to yield final concentrations of 55, 75, 95, 115, 135 µg/ml (paracetamol), and 7, 9, 11, 13, 15 µg/ml (diclofenac).

Sample treatment

For the analysis of pharmaceutical preparations, 20 tablets were pulverized and an average weight of a single tablet, equivalent to 500 mg paracetamol and 50 mg diclofenac, was weighed, transferred to a 100 ml volumetric flask and diluted with buffer to the mark. The content of the flask was ultrasonicated for 10 minutes, and the solution was filtered through a 0.45 µm syringe filter. 1 ml filtrate was transferred to a 10 ml flask and diluted with buffer to the mark.

Blank sample: Working buffer solution

CE Method Development

Fixed electrophoresis conditions are as follows: uncoated fused-silica capillary column of a total length of 50 cm (effective length 41.5 cm) [8]; capillary temperature: 250C [8]; injection pressure 35 mbar and injection time 4s, wavelength 276 nm [1].

Experiment to select buffer solution type, buffer solution concentration, buffer solution pH, and voltage on capillary ends.

Validation of the method

The method was validated according to AOAC 2016 (Association of Official Analytical Chemists) and ICH 2005 guidelines (International Conference on Harmonisation) about the validation of analytical procedures including the following criteria: system compatibility, specificity, linearity range, precision, and accuracy.

Processing statistics are calculated based on Microsof Excel 2016 software.

3. RESULTS

3.1. CE Method Development

The experiments were conducted to select buffer type and concentration, pH, applied voltage, combined with the fixed parameters: the uncoated fused-silica capillary column of a total length of 50 cm (effective length 41.5 cm) [8], the inner diameter of 50 µm, sample injection mode 35 mbar for 4s, capillary temperature: 250C [8], detection wavelength 276 nm [1].

The optimum conditions determined were as follows: sodium tetraborate buffer solution 50 mM, pH = 9, the voltage applied 30 kV.

3.2. Validation of the method

3.2.1. System suitability

Capillary electrophoresis system suitability was determined by repeated injection of 6 standard solutions of paracetamol 100 µg/ml and diclofenac 10 µg/ml. Results of the capillary electrophoresis system suitability are presented in Table 1.

Table 1. Results of capillary electrophoresis system suitability (n = 6)

Serial Number	Paracetamol				Diclofenac				R_S
	T_R *(min)*	S *(mAu.s)*	N	A_s	T_R *(min)*	S *(mAu.s)*	N	A_s	
1	2.07	8.10	12744.00	1.00	3.08	1.97	12738.00	0.90	10.87
2	2.08	7.92	13512.00	1.00	3.09	2.03	11955.00	0.90	10.95
3	2.08	7.85	13057.00	1.00	3.08	2.00	12880.00	0.90	11.04
4	2.10	7.84	13512.00	1.00	3.19	2.01	9474.00	0.90	10.65
5	2.11	7.86	13496.00	1.00	3.22	1.92	9634.00	0.90	10.83
6	2.10	7.94	13588.00	1.00	3.16	2.03	11124.00	1.00	11.10
Average	2.09	7.92	13318.17	1.00	3.14	1.99	11300.83	0.92	10.91
RSD%	0.76	1.21	2.55	0.00	1.96	2.19	13.21	4.45	1.49

n is the number of experiments

TR: Retention time; S: Peak area; N: Plate theory; R: Resolution; As: Asymmetry

According to Table 1, the relative standard deviation of retention time and peak area are within the permissible range (<3.0%) [1]. This proves that the capillary electrophoresis system is suitable for the simultaneous determination of paracetamol and diclofenac.

3.2.2. Specificity

Conduct analysis of the blank, the mixed standard solution, the Zengesic and Ripangesic samples. Electropherograms are shown in *Figure 1.*

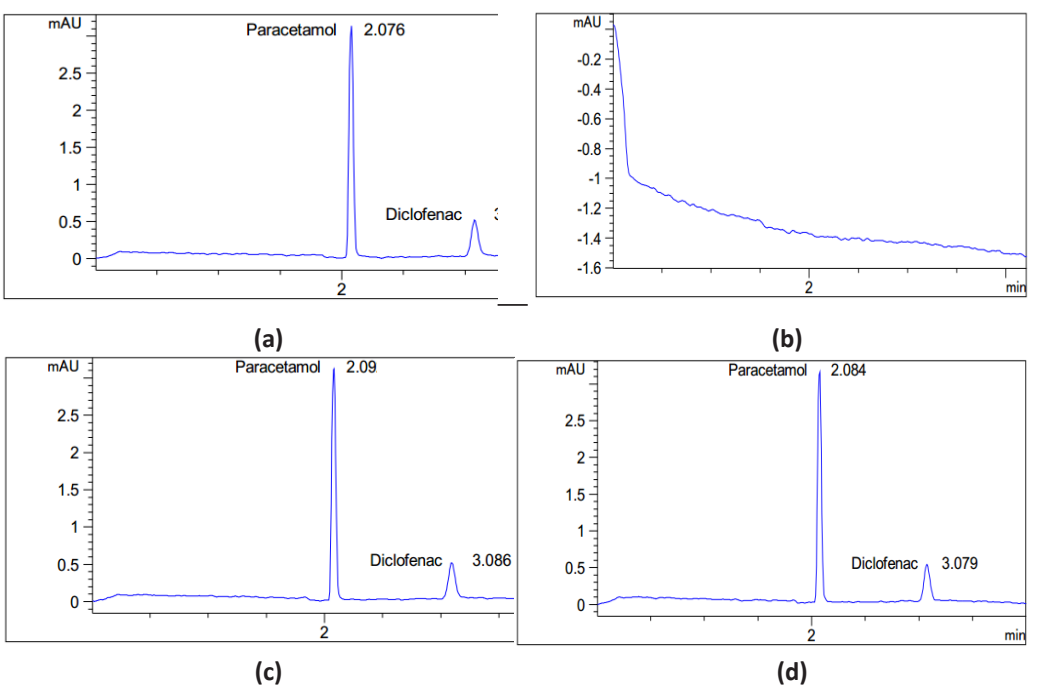

Figure 1. Electropherogram

a. Blank sample; b. Standard mixture solution; c. Zengesic tablet; d. Ripangesic tablet

The selectivity of the method was tested by comparing the electropherogram of standard solutions and commercial pharmaceutical product solutions containing an equivalent concentration of analytes. No interference occurred from excipients with the drug peaks of the studied analytes during the analysis of formulation samples. Electropherogram showed no peaks of paracetamol and diclofenac in the blank. The sample had a characteristic paracetamol peak at 2.0 minutes and a diclofenac peak at 3.0 minutes; which has

similar retention time to those in the standard mixture solution. The UV spectroscopy test of the standard solution by PDA detector and the purity index in Figure 2 showed that the two peaks having high purity with the purity of paracetamol and diclofenac are both 0.999. The above results showed that the method has good specificity.

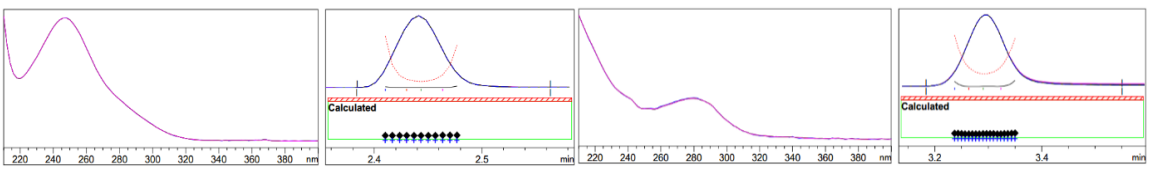

Figure 2. UV spectrum and peak purity
(a) Paracetamol; (b) Diclofenac

3.2.3. Linearity range

The stock solutions were diluted with buffer solution to secondary standard solutions with an accurate concentration of about 55 - 135 µg/ml for paracetamol and 7-15 µg/ml for diclofenac.

The linear relationships between the concentration of the two analytes and the corresponding peak height were investigated under the optimum separation conditions. The regression equations and correlation coefficients are presented in Table 2. The calibration curves were plotted for paracetamol and diclofenac in Figure 3.

Table 2. Linearity regression data for the CE separation of paracetamol and diclofenac

Paracetamol	**C (µg/ml)**	54.45	74.25	94.05	113.85	133.65	**y = 0.0846x + 0.015 R^2 = 0.9978**
	A (mAU.s)	4.59	6.46	7.84	9.56	11.42	
Diclofenac	**C (µg/ml)**	6.97	8.96	10.95	12.94	14.93	**y = 0.1926x + 0.048 R^2 = 0.9968**
	A (mAU.s)	1.35	1.83	2.15	2.54	2.91	

The linear regression data for the calibration curves showed good linear relationships between peak area and concentration. The correlation of coefficient (r) was found to be 0.9989 and 0.9984 for paracetamol and diclofenac, respectively.

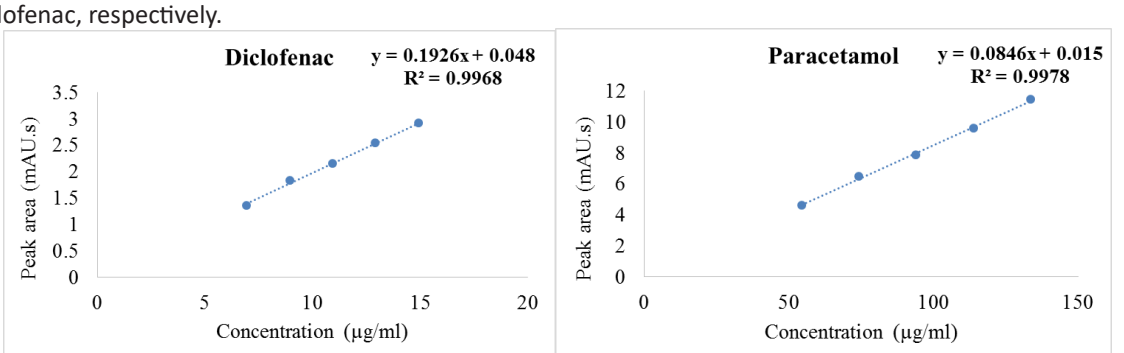

Figure 3. Calibration curve of paracetamol and diclofenac

3.2.4. Precision

To assess the repeatability of the method, 6 separate weighing samples of Zengesic film-coated tablets were analyzed by the proposed methods. The samples were prepared using the procedure given in *Section 2.2*. The results of repeatability assessment of CE method were presented in *Table 3.*

Table 3. The results of the repeatability assessment of the CE method

S.No	**Weight of sample taken (mg)**	**Paracetamol**		**Diclofenac**	
		S (mAu.s)	**Content %**	**S (mAu.s)**	**Content %**
1	671.90	8.53	100.57	1.97	99.67
2	671.70	8.25	97.32	1.94	98.39

3	672.60	8.27	97.38	2.03	102.75
4	671.10	8.40	99.16	2.00	101.19
5	671.80	8.42	99.32	1.98	100.30
6	671.90	8.37	98.51	1.96	98.85
Average			98.71		100.19
RSD%			**1.26**		**1.60**

As can be seen from Table 3, the present method is precise in all sample applications which meet the guideline on validation of analytical methods by AOAC with relative standard deviation RSD for paracetamol less than 1.3% (1.26%) and RSD for diclofenac less than 1.9% (1.60%) [5].

3.2.5. Accuracy

To study the accuracy of the developed method, a recovery study was carried out by using the standard addition method at two different levels for Zengesic film-coated tablets and then calculated the % recoveries. The amounts of the added standard were approximately 10%, 20%, and 30% of the total amounts of each active ingredient in the tablet. The recovery study was performed three times at each level and analyzed by the proposed methods, the results were shown in Table 4.

Table 4. % Recovery of paracetamol and diclofenac in combined dosage form

Name of compound	% Added	Serial Number	Amount added	Peak area (mAU.s)	% recovery	Average	RSD%
Paracetamol	**10%**	**1**	49.70	9.12	98.01	98.66	0.74
		2	50.00	9.29	99.46		
		3	49.60	8.98	98.51		
	20%	**1**	99.60	9.99	100.37	100.16	0.80
		2	100.50	10.14	99.28		
		3	100.60	9.87	100.84		
	30%	**1**	151.10	10.73	100.84	99.98	0.77
		2	157.20	10.80	99.36		
		3	152.10	10.86	99.72		
Diclofenac	**10%**	**1**	5.20	2.19	101.86	100.43	1.58
		2	5.00	2.19	98.73		
		3	5.10	2.14	100.71		
	20%	**1**	9.90	2.36	99.06	100.53	1.45
		2	9.70	2.37	100.56		
		3	10.00	2.33	101.97		
	30%	**1**	16.20	2.55	98.10	99.92	1.61
		2	17.10	2.60	100.52		
		3	15.10	2.57	101.15		

Average recoveries for paracetamol were from 98.01 to 100.84% and diclofenac were from 98.10 to 101.97%. That are between the AOAC recommended ranges 98 – 102% for both paracetamol and diclofenac [5].

3.3. Application

The developed method has been applied for the determination of paracetamol and diclofenac from pharmaceutical preparations. The samples were prepared using the procedure given in Section 2.2. The resulting contents of the analytes in the samples are listed in Table 5.

Table 5. Assay results of paracetamol and diclofenac in pharmaceutical preparations (n = 3)

Commercial samples	S.No	Paracetamol			Diclofenac natri		
		Peak area (mAU.s)	Content (%)	Average ±SD	Peak area (mAU.s)	Content (%)	Average ±SD
Zengesic	1	8.43	100.06	99.32 ± 0.71	1.94	98.30	99.18 ± 0.86
	2	8.31	98.64		1.98	100.02	
	3	8.36	99.28		1.96	99.23	
Ripangesic	1	8.43	100.19	99.59 ±0.55	1.98	100.26	99.36 ± 0.95
	2	8.37	99.47		1.96	99.45	
	3	8.34	99.11		1.94	98.37	

4. DISCUSSION

In the world, there have been a number of studies on the simultaneous quantitative process of paracetamol and diclofenac by different methods, HPLC method was performed by B Gowramma (2010) [7], a derivative spectroscopy method was also proposed by Rajesh Sharma in 2010 [10]. In Vietnam, Le Thi Y Nhi used a derivative spectroscopy method to quantify paracetamol and diclofenac simultaneously [2]. In general, the spectroscopic method is simple, easy to implement, but complicated at the phase of creating a colored reaction; while HPLC method usually gives good separation results, high accuracy and sensitivity, but has the high cost of sample analysis and solvent-consuming.

In the past few years, capillary electrophoresis (CE) has emerged to be an important tool in the analysis, due to its separation efficiency, low amount of sample and reagent consumption, speed of analysis, and applications to a wider selection of analytes. In 2010, this method was applied by Amber Solangi and colleagues to separate, quantify, and validate simultaneously ceftriaxone, ceftizoxime, paracetamol, and diclofenac sodium in pharmaceutical formulations and human blood serum.

The separation process uses a 50 mM sodium tetraborate buffer, pH = 9, fused silica capillary length 57 cm (effective 50 cm, ID = 75 µm), the voltage applied to both ends of the capillary 30 kV, capillary temperature 250C, a sample injection pressure of 35 mbar for 4s and detected at 214 nm. Each analysis time lasts more than 12 minutes (preconditioning time for 6 minutes, sample analysis time for 8 minutes) [11].

Inheriting and continuing to improve the method of simultaneous determination of paracetamol and diclofenac by capillary zone electrophoresis, our study used a capillary column of a total length of 50 cm (41.5 cm effective length), 50 µm ID, the voltage applied to both capillary ends 30 kV, sodium tetraborate buffer solution 50 mM (pH = 9), sample analysis time was shorter (preconditioning time for 6 min, sample analysis time for about 3.5 min). This might be explained by the lower number of analytes and the shorter capillary column selected for the separation in our study. Furthermore, the use of CE Agilent 7100 system with several advantages such as wavelength accuracy of DAD detector lower than 1 nm, fast sampling rate (40 Hz), has improved the accuracy of our study (paracetamol 0.74 – 0.80%; diclofenac 1.45 – 1.61%) compared with Amber Solangi's study (paracetamol 1.8 – 2.1; diclofenac 2.0% – 2.1%).

In 2016, Nguyen Huu Tien and colleagues simultaneously determined paracetamol and diclofenac in pharmaceutical formulations by HPLC, the study used HiQ Sil C18 chromatography column (250 x 4.6mm, 3µm), mobile phase including MeOH - Acetate buffer pH 5.0 (80: 20, v/v), flow rate 0.7 ml/min, and the detection wavelength was at 281 nm. Compared with the above method, our method has a shorter analysis time (sample run time by CE is about 3.5 minutes compared to 5.2 minutes by HPLC), a large number of theoretical plates (CE: 105 - 106; HPLC: 103 - 4x104) leads to excellent separation efficiency, narrower peaks, and better resolution. The analytes moved evenly in the capillary under the influence of the electrophoretic mobility create moving bands instead of parabolic moving regions like in HPLC. However, the accuracy of this method is lower than that of the HPLC method. For CE, the two parameters to evaluate precision are peak area for quantitative analysis and retention time for qualitative analysis. The precision of the peak area is assessed by the RSD parameter. The inaccuracy of the peak area was influenced by two main factors,

the amount of sample injected and the capillary effect. We limited this effect by optimizing capillary activation for the first use, rinsing capillaries at the beginning of the day, between injections, and at the end of the day. Although the RSD parameter of peak area obtained by the CE method (paracetamol 1.26%; diclofenac 1.60%) was lower than the HPLC method (paracetamol 1.08%; diclofenac 1.30%), it still met the requirement of precision of AOAC 2016.

4. CONCLUSIONS

A rapid and simple capillary electrophoresis method has been developed for the simultaneous determination of paracetamol and diclofenac in pharmaceutical formulations. A 50 mM sodium tetraborate background electrolyte solution (pH 9.0) was found to be suitable for separation. An uncoated fused - silica capillary of a total length of 50 cm (effective length 41.5 cm) was used for separation. All the analytes were completely separated within 3.5 min at an applied voltage of 30 kV, sample injection mode 35 mbar for 4s, and detection was performed at 276 nm. The method had systematic suitability, high selectivity, suitable linear range, precision, and accuracy in accordance with AOAC 2016 standards. The proposed method has been successfully applied to the analysis of paracetamol and diclofenac in the pharmaceutical preparations on the market.

REFERENCES

1. Ministry Of Health (2017), Vietnamese Pharmacopoeia V Part, Medical Publishing House one member Company Limited, HaNoi.

2. Le Thi Y Nhi (2015), Simultaneous determination of paracetamol and diclofenac sodium in tablets by derivative spectroscopy, Graduation thesis of university pharmacist 2010-2015, Hue University of Medicine and Pharmacy.

3. Nguyen Huu Tien, Nguyen Viet Khan, Pham Viet Ty (2016), "Simultaneous determination of paracetamol and diclofenac sodium in tablets by HPLC", Journal of Science and Education, Hue University of Education 3(39), 86-92.

4. Agilent Technologies Inc (2015), Agilent 7100 Capillary Electrophoresis System.

5. AOAC Official Methods of Analysis (2016), Guidelines for Standard Method Performance Requirements, Appendix F. pp. 1-18.

6. Curatolo, Michele Sveticic, Gorazd (2002), "Drug combinations in pain treatment: a review of the published evidence and a method for finding the optimal combination", *Best Practice & Research Clinical Anaesthesiology*. 16(4), 507-519.

7. Gowramma, B (2010), "A validated RP-HPLC method for simultaneous estimation of paracetamol and diclofenac potassium in pharmaceutical formulation", *International Journal of Chemtech Research*. 2(1), 676-680.

8. Kaster, JA (1993), "Essential guides to method development in capillary electrophoresis", *Nature*. 384(17), 19.

9. Roy, D Altman (2004), "A rationale for combining acetaminophen and NSAIDs for mild-to-moderate pain", *Clinical and Experimental Rheumatology.* 22(1), 110.

10. Sharma, Rajesh (2010), "Spectrophotometric methods for simultaneous estimation of paracetamol and diclofenac sodium in combined dosage form by application of hydrotropic solubilization", *Journal of Pharmaceutical Sciences and Research*. 2(12), 821.

11. Solangi, Amber (2010), "Determination of ceftriaxone, ceftizoxime, paracetamol, and diclofenac sodium by capillary zone electrophoresis in pharmaceutical formulations and in human blood serum", *Turkish Journal of Chemistry*. 34(6), 921-934.

.