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(PAPER ID : 350)

MICELLAR ELECTROKINETIC CHROMATOGRAPHY METHOD FOR KETOROLAC ANALYSIS IN PHARMACEUTICAL SAMPLE

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ABSTRACT. A micellar electrokinetic chromatography (MEKC) method for quantitative determination of ketorolac, a non-steroidal anti-inflammatory drug (NSAID) in tablet and water samples is reported. Optimization of MEKC conditions were carried out by changing the sodium dodecyl sulfate (SDS) concentration, borate buffer (pH 9.3) concentration, and applied voltage. The optimized MEKC method using 30 mM SDS, 10 mM borate buffer (pH 9.3) and 30 kV applied voltage has been successfully applied for the determination of ketorolac in tablet sample with analysis time less than 5 min. The average recovery of ketorolac in tablet sample was 97.56% (*RSD* = 0.75%). On-line preconcentration technique in MEKC was then investigated to increase detection sensitivity of ketorolac. A sensitivity enhancement factor of 6-fold was achieved using the normal stacking mode (NSM)-MEKC. The average recovery of ketorolac in spiked tap water sample was 102.16% (*RSD* = 0.06%). The present NSM-MEKC method was simple, short analysis time, high accuracy and environmentally friendly.

INTRODUCTION

Ketorolac, a non-steroidal anti-inflammatory drug (NSAID), is primarily used for the treatment of postoperative pain and has been shown to have opioid-sparing effects and reduces opioid-related side effects. Ketorolac tromethamine is the first NSAID approved for parenteral use. It is used for a variety of clinical indications, but is mainly administered for the management of postoperative pain. It can also be used for treatment of cancer-related pain, for pain after cesarean delivery, and in the emergency department for treatment of migraine headaches, renal colic, musculoskeletal pain, and sickle cell crisis. Ketorolac has been used safely and effectively in select pediatric populations but at present is not recommended for use in children under the age of 17 (Nalini Vadivelu et al., 2017). Chemical structure of ketorolac is shown in figure 1. Several methods for determination of ketorolac have been reported, such as spectroscopic (Settaluri and Division, 2015; Narayana et al., 2015), liquid chromatography (Sunil et al., 2017; Khairnar and Anantwar, 2014) (Selvadurai Muralidharan and Subramani, 2013), and capillary electrophoresis methods (Alba Macia et al., 2007; Orlandini et al., 2004).

Micellar electrokinetic chromatography (MEKC), a hybrid of electrophoresis and chromatography introduced by Terabe in 1984 (Terabe et al., 1984), has become popular as a powerful technique for improving separation efficiency not only of neutral analytes but charged ones by using a capillary electrophoresis (CE) instrument without any alteration (Rezende et al., 2020; Ciura et al., 2020; Ko et al., 2019; Fan Gao and Xiao-Fei, 2019; Michael et al., 2013; Silva, 2011). In MEKC, an anionic surfactant is used as a pseudostationary phase

that corresponds to the stationary phase in conventional chromatography and the surrounding aqueous phase to the mobile phase. The separation principle of analytes is based on their differential partitioning between the aqueous phase and the micellar phase.

Poor concentration sensitivity in CE is mainly due to the small amount of sample injected (1 – 10 nL) and a short optical pathlength equal to the capillary diameter, for absorbance detectors (Peng et al., 2020; Roberto Gotti et al., 2019; Marta Gładysz et al., 2018; Hermawan et al., 2011). One possible solution is to use highly sensitive detectors for laser-induced fluorescence or electrochemical measurements. Another solution is to use extended optical path length cells such as a bubble cell or Z-type cell, which can increase sensitivity with a minimal decrease in resolution. However, all these methods require rather expensive and somewhat complex hardware or time consuming procedures. A more promising choice for increasing concentration sensitivity is on-line sample preconcentration, in which a sample plug longer than normal is injected and focused inside the capillary before separation. Either pressurized (also known as hydrodynamic) or electrokinetic injection can be used. Sample stacking and sweeping are known as two on-line sample preconcentration techniques for enhancement of the concentration sensitivity in MEKC (Lin et al., 2016; Minglei Wu et al., 2015; Mal et al., 2011; Wan Ibrahim et al., 2010).

The aim of the present work was to develop the MEKC method for the quantitative determination of ketorolac in pharmaceutical (tablet) sample. The proposed MEKC method for ketorolac analysis is simple, rapid analysis, high accuracy and requires minimal organic solvent used (environmentally friendly).

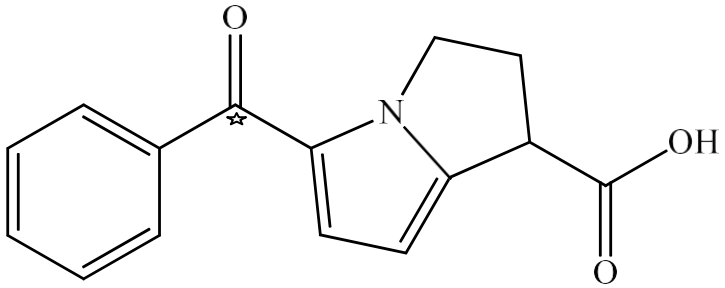

Figure 1. Chemical structure of ketorolac

METHODS

Materials

Ketorolac was purchased from Sigma-Aldrich (St Louis, MO, USA). Sodium dodecyl sulphate (SDS) was purchased from Fischer Chemicals (Loughborough, Leics, UK). Sodium hydroxide and sodium tetraborate anhydrous were purchased from Merck (Darmstadt, Germany). Organic solvents (HPLC grade) were purchased from J.T Baker (Pennsylvania, USA). Deionized water (DI) was purified by Millipore Simplicity (Simpak®2) (Barnstead, USA). Working standard solutions were prepared by appropriate dilution of standard stock solution with methanol. The standard stock was stored in refrigerator until needed.

Instrumentation

A CE system from Agilent Technology (7100, Waldbronn, Germany) equipped with DAD operating at 320 nm wavelength was used for the analysis. Uncoated fused-silica capillary of 50 µm inner diameter (I.D.) with a total length of 64.5 cm (56 cm to detector) was used for the separation process. Analytical data were collected from CE system (Chromatography Station CSW 1.7). A 30 min of conditioning process with 1.0 M NaOH was performed for every new capillary before it was used. The new capillary was then equilibrated with DI water for 30 min, followed by 0.1 M NaOH for 15 min and DI water for 15 min, and finally with the BGE solution for 10 min. In all cases, hydrodynamic injection was used at 50 mbar for 5 s (5 nL sample volume) at the capillary inlet to optimize the separation. The capillary was pre-conditioned for 2 min with the BGE solution and post-conditioned with 0.1 M NaOH and DI water for 2 min between every analysis.

Validation Procedure

The performance of the method was examined in terms of linearity, repeatability, limit of detection (LOD), limit of quantification (LOQ) and sensitivity enhancement factor (SEF) or increase in detection sensitivity. Linearity of the optimized method was assessed by construcing the calibration curve of peak area (n

= 3) against with the concentration of standard ketorolac (at the linear range). The repeatability was recognized in term of relative standard deviation (%RSD, n = 3). The limit of detection (LOD) and limit of quantification (LOQ) were determined by the calibration curve along with the signal to noise ration (S/N) as 3 and 10, respectively.

RESULTS AND DISCUSSION

Optimization is carried out with several parameters to get the optimum conditions from MEKC. Some of the parameters used include the effect of borate buffer concentration, the effect of SDS concentration, and the effect of applied voltage has been observed. The optimum results from these parameters are used to validate and determine the levels of analyte in the sample.

The effect of borate buffer concentration (pH 9.3) was evaluated in this study, ranging from 5 to 15 mM. A pH 9.3 was selected in this study in order to increase the lifetime of the capillary since higher pH degraded the silica inner wall of the capillary rapidly (Hermawan et al., 2011). Using borate buffer of low-ionic strength resulted in the generation of low currents. As can be seen in Figure 2, it was found that increase in the concentration of borate buffer from 5 to 15 mM caused increase in the migration time of ketorolac. A 10 mM borate buffer was selected for optimal concentration since it yielded higher of peak height than 5 mM borate buffer, and faster migration time than 15 mM borate buffer. This buffer concentration was employed in all subsequent investigations.

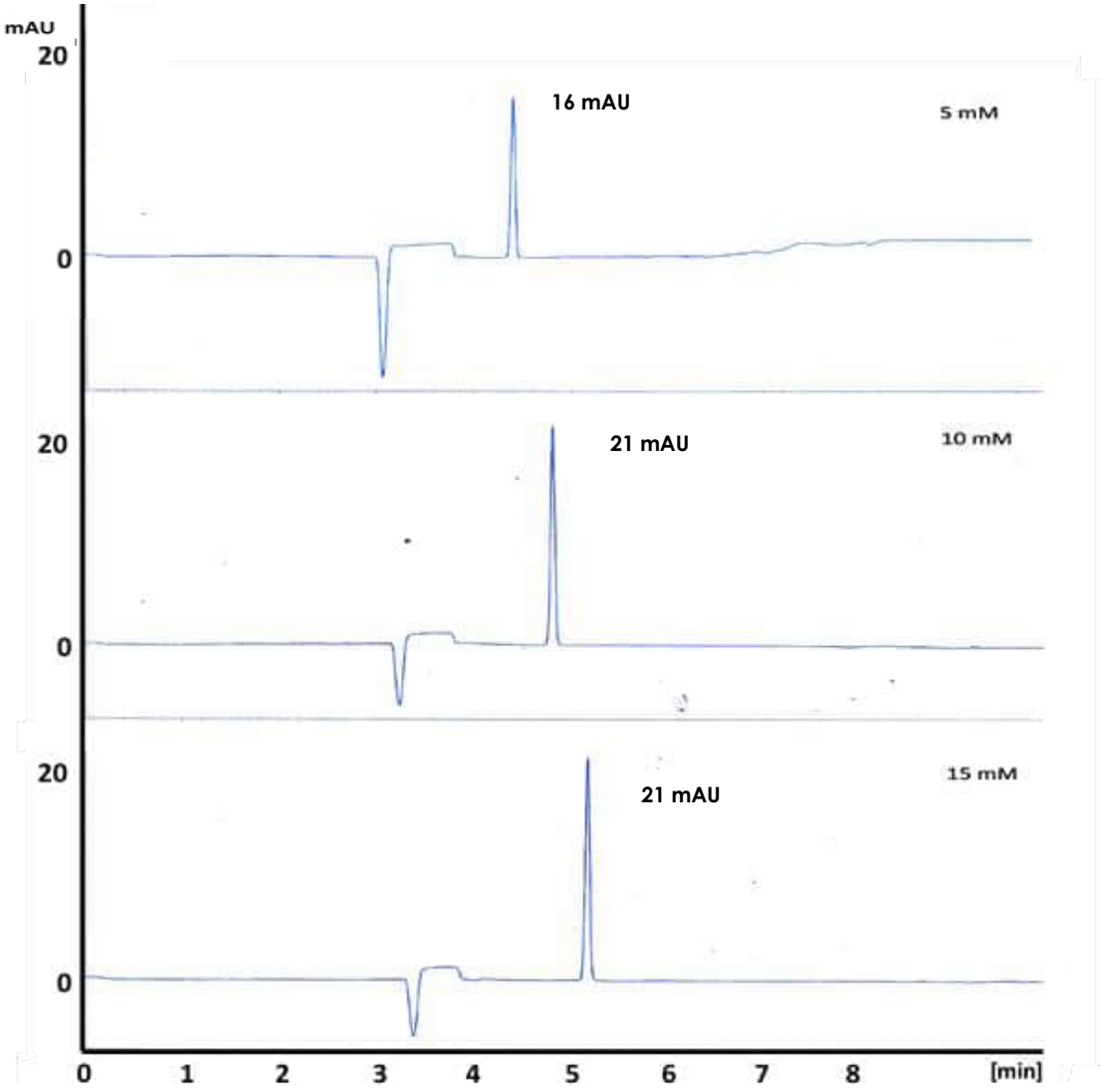

Figure 2. Electropherogram of ketorolac with different concentration (mM) of borate buffer (pH 9.3)

The effect of SDS concentration was also evaluated in this study, ranging from 25 to 35 mM (with 10 mM borate buffer concentration pH 9.3). As can be seen in Fig. 3, It was found that the migration times of the ketorolac was not significantly increased. A 30 mM SDS was selected for optimal concentration since it yielded highest of peak height. This concentration was employed in all subsequent investigations.

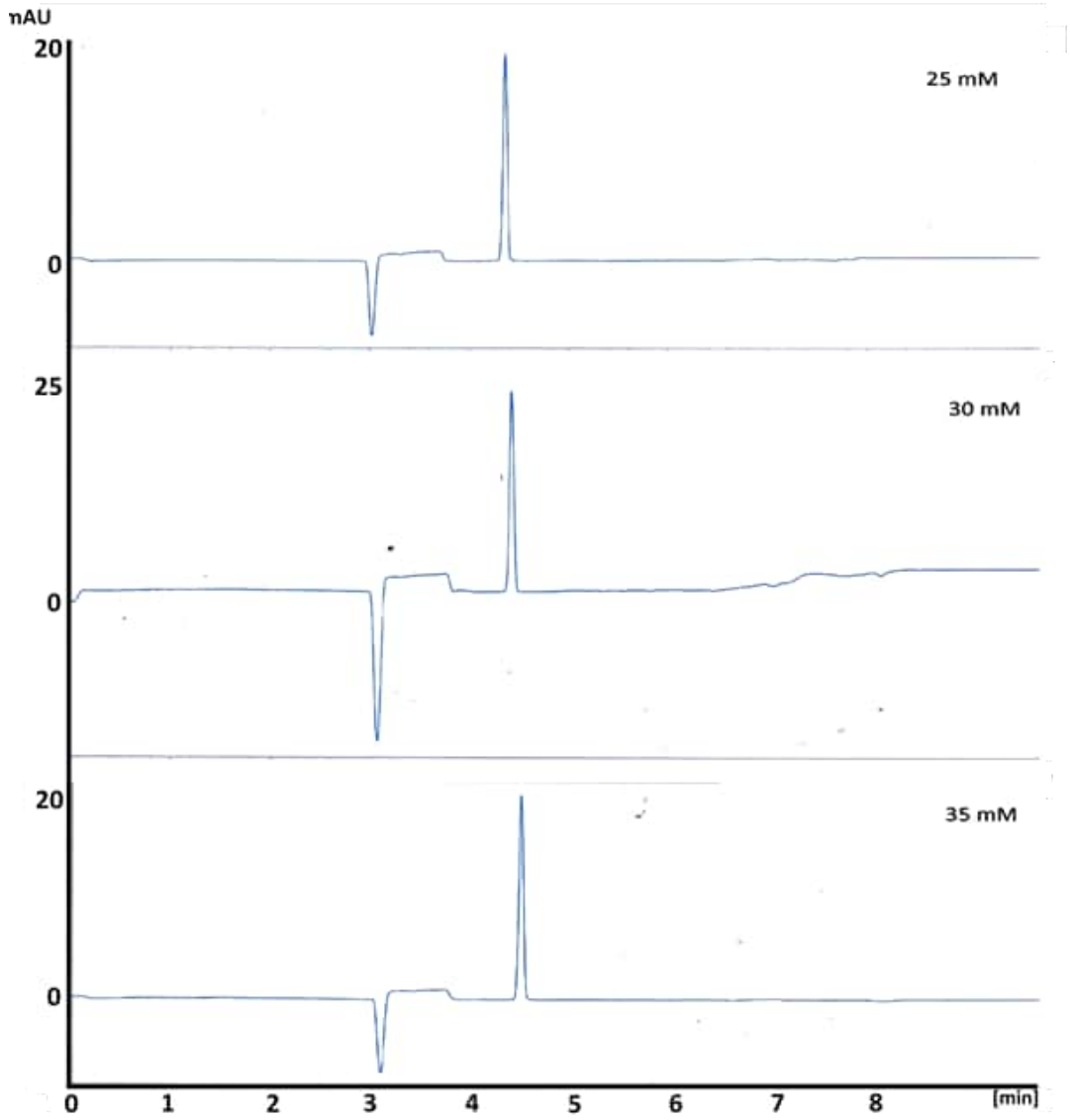

Figure 3. Electropherogram of ketorolac with different concentration of SDS (mM)

In addition, effect of applied voltage was also optimized for the MEKC method and it was found that the best applied voltage was obtained at 30 kV. Lower applied voltage than 30 kV was found to increase migration time. The electropherogram is shown in Figure 4. Since shorter analysis time is more applicable during analysis, 30 kV was chosen as the best applied voltage. Applied voltage of 30 kV was employed in all subsequent investigations by MEKC.

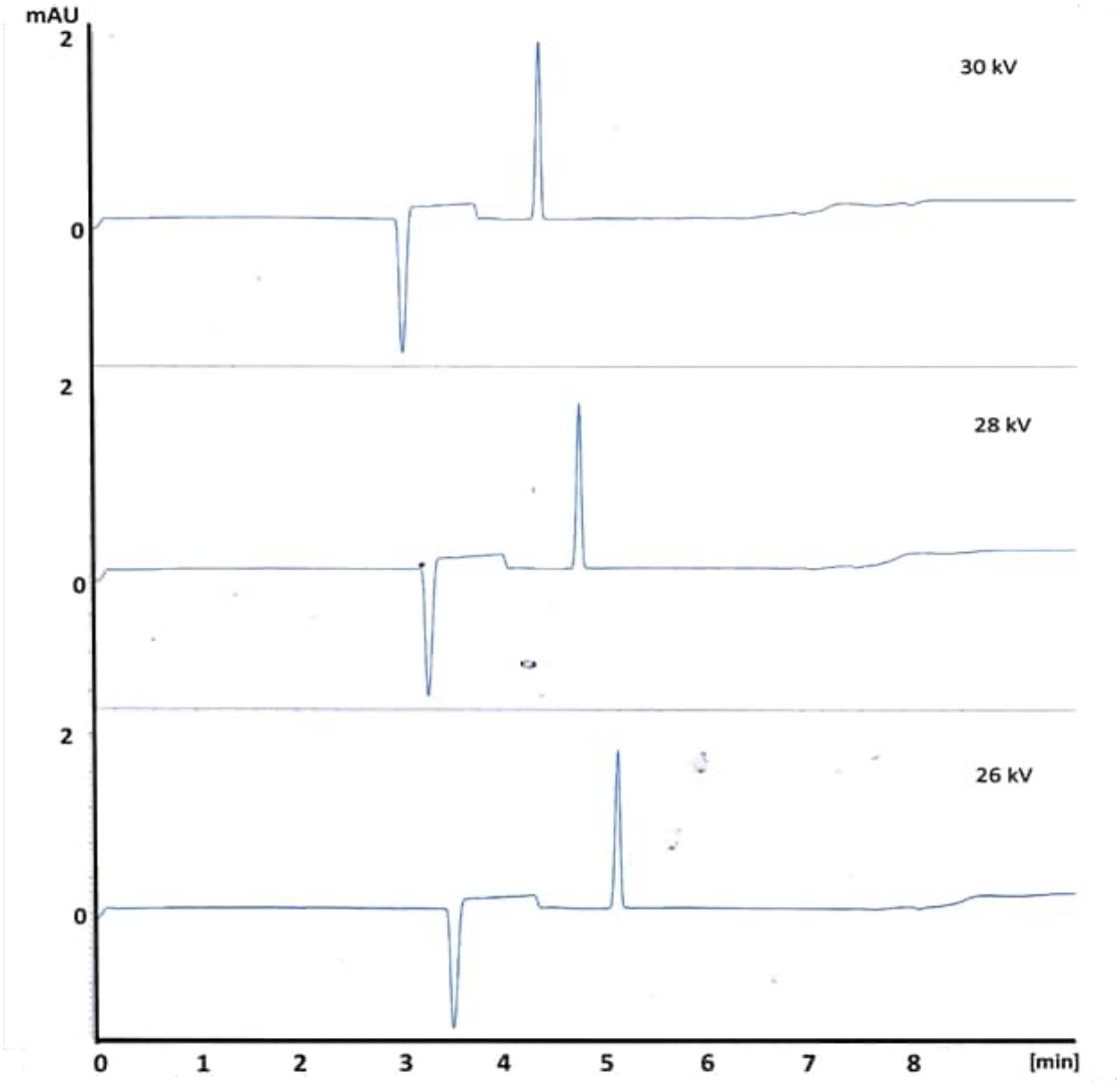

Figure 4. Electropherogram of ketorolac with different applied voltage (kV)

The performance of the method was examined in terms of linearity, reproducibility, limit of detection (LOD), and limit of quantification (LOQ). Linearity of the optimized method was assessed by constructing the calibration curve of average peak areas (n = 3) against the concentration of ketorolac standards (at the linear range). The reproducibility in the migration time, peak area, and peak height were recognized in terms of the relative standard deviation (RSD %, n = 3). The LOD was determined from the calibration curve along with the signal-to-noise ratio (S/N) as 3 and the LOQ as S/N = 10. The excellent linearity was obtained in the range of concentration from 50 to 200 µg/mL with good linearity (r^2 = 1.00). The LOD and LOQ of ketorolac standard were determined by the calibration curve along with signal to noise (S/N) as 1.04 µg/mL and 3.46 µg/mL, respectively.

This result is better than the previous study on the determination of ketorolac by the liquid chromatography method (Sunil et al., 2017), in term of linearity and rapid analysis time. In their study, range of concentration from 50 to 150 µg/mL (correlation coefficient (r) of 0.999) and retention time within 6 min. Electropherogram of ketorolac standard by the optimized MEKC method is shown in Figure 5 (A), with migration time of ketorolac within 5 min. In addition, the electropherogram of ketorolac in tablet sample by the optimized MEKC method is presented in Figure 5 (B). The average recovery of ketorolac in tablet sample was 97.56% (*RSD* = 0.75%, n=3).

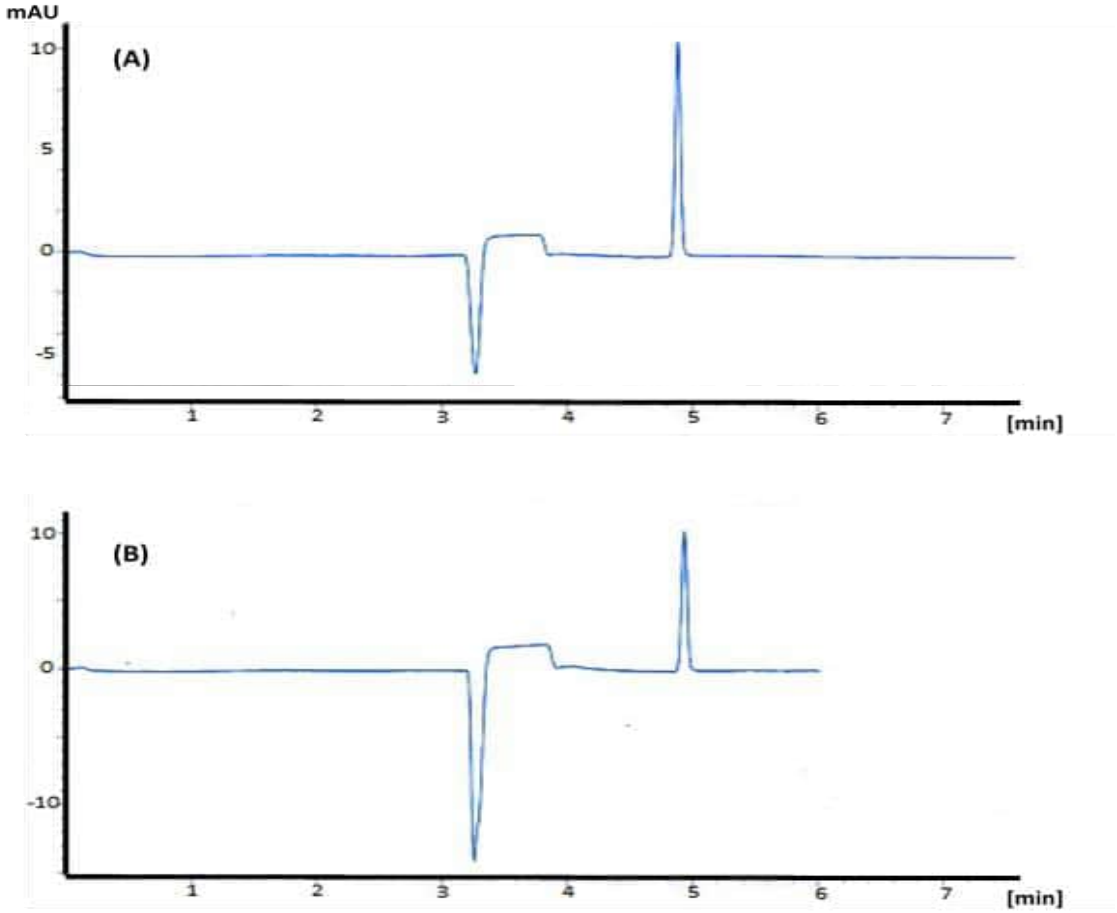

Figure 5. Electropherogram of (A) 100 µg/mL ketorolac standard; and (B) ketorolac in tablet sample; by the optimized MEKC method

CONCLUSIONS

Application of micellar electrokinetic chromatography (MEKC) method for the analysis of ketorolac in tablet sample is reported. The optimized MEKC method using 30 mM SDS, 10 mM borate buffer (pH 9.3) and 30 kV applied voltage was applied for the determination of ketorolac in tablet sample with average recovery of ketorolac in tablet sample was 97.56% (*RSD* = 0.75%). The run time was within 5 min which enabled a rapid quantification of many samples in routine and quality control analysis of ketorolac. This result can be used as a preliminary study for ketorolac analysis.

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REFERENCES

Alba, M., Francesc, B., Marta, C., and Carme, A., 2007. Aguilar, Capillary Electrophoresis for the Analysis of Non-Steroidal Anti-Inflammatory Drugs. *Trends in Analytical Chemistry*, 26, 133–153. doi: https://doi.org/10.1016/j.trac.2006.11.011.

Ciura, K.K., Hanna, D., Szymon, K., Piotr, B., Mariusz, B., and Tomasz., 2020. Biopartitioning Micellar

Electrokinetic Chromatography – Concept Study of Cationic Analytes. *Microchemical Journal*, 154, 104518 doi: https://doi.org/10.1016/j.microc.2019.104518.

Fan, G., Xiao-Fei, W., and Bo, Zhang., 2019. Research and Application Progress of Micellar Electrokinetic Chromatography in Separation of Proteins. *Chinese Journal of Analytical Chemistry*, 47, 805–813. doi: https://doi.org/10.1016/S1872-2040(19)61163-1.

Hermawan, D., Wan Ibrahim, W.A., and Sanagi, M.M. (2011). *Chiral Triazole Fungicides Separation by Micellar Electrokinetic Chromatography*. Saarbrücken: Lambert Academic Publishing.

Hermawan, D., Mohd Yatim, I., Ab Rahim, K., Sanagi, M.M., Wan Ibrahim, W.A., Aboul-Enein, H.Y. 2013. Comparison of HPLC and MEEKC for Miconazole Nitrate Determination in Pharmaceutical Formulation. *Chromatographia*. 76, 1527–1536. https://doi.org/10.1007/s10337-013-2390-1

Hung-Ju, L., Kun-Pin, H., Shyh-Shin, C., Hwang-Shang, K.W., and Shou-Mei., 2016. Determination of Deferasirox in Human Plasma by Short-end Injection and Sweeping with a Field-Amplified Sample Stacking and Micellar Electrokinetic Chromatography. *Journal of Pharmaceutical and Biomedical Analysis*, 131, 497–502. doi: https://doi.org/10.1016/j.jpba.2016.06.042.

Khairnar, D.A., Anantwar, C.S., Chaudhari., and Sanjay, P., 2014. Method Development and Validation of Ketorolac Tromethamine in Tablet Formulation by RP-HPLC Method. *International Journal of Pharmaceutical Sciences and Research*, 5, 3696–3703. doi: https://doi.org/10.13040/IJPSR.0975-8232.5(9).3696-03.

Ko, H.Y., Yi-Hui, L., Chi-Jen, S.C., and Yen-Ling., 2019. Determination of Phenylenediamines in Hair Colors Derivatizated with 5-(4, 6-dichlorotriazinyl) aminofluorescein via Micellar Electrokinetic Chromatography. *Journal of Food and Drug Analysis*, 27, 825–831. doi: https://doi.org/10.1016/j.jfda.2019.02.005.

Mal, Z.G., Petr, E., and Petr, Boc., 2011. Contemporary Sample Stacking in Analytical Electrophoresis. *Electrophoresis*, 32, 116–126. doi: https://doi.org/10.1002/elps.201000327.

Marta, G., Małgorzata, K., Michał, W., and Paweł, K., 2018. The Increase of Detection Sensitivity of Micellar Electrokinetic Capillary Chromatography Method of Stamp Pad inks Components by Applying a Sample Stacking Mode for the Purpose of Questioned Document examination. *Talanta*, 184, 287–295. doi: https://doi.org/10.1016/j.talanta.2018.02.091.

Michael, E., El-Kommos., Niveen, A.M., Ahmed, F., and Abdel, H., 2013. Selective Micellar Electrokinetic Chromatographic Method for Simultaneous Determination of some Pharmaceutical Binary Mixtures Containing Non-Steroidal Anti-Inflammatory Drugs. *Journal of Pharmaceutical Analysis*, 3, 53–60. doi: . https://doi.org/10.1016/j.jpha.2012.07.005.

Minglei, W., Fan, G., Yi, Z., Guan, W., Qingjiang, W., and Hui, L., 2015. Sensitive Analysis of Antibiotics via Hyphenation of Field-Amplified Sample Stacking with Reversed-Field Stacking in Microchip Micellar Electrokinetic Chromatography. *Journal of Pharmaceutical and Biomedical Analysis*, 103, 91–98. doi: https://doi.org/10.1016/j.jpba.2014.11.004.

Nalini, V., Daniel, C.H., Erik, M.B., Gregory, J., Alice, K., Alan, D., Dora, B., Daniel, J., and Inderjeet., 2017. Ketorolac, Oxymorphone, Tapentadol, and Tramadol: A Comprehensive Review, Anesthesiol. *Anesthesiology clinics*, 35, 1–20. doi: https://doi.org/10.1016/j.anclin.2017.01.001.

Narayana., Nasrin, B.S.I., and Badiadka., 2015. Spectrophotometric Determination and Spectroscopic Studies on Schiff Base and Charge Transfer Complex of Ketorolac Tromethamine. *Journal of Analytical Science and Technology*, 5, 1–12. doi: https://doi.org/10.1186/s40543-015-0075-0.

Orlandini, S., Fanali, S., Furlanetto, S., Marras, A.M., and Pinzauti, S., 2004. Micellar Electrokinetic Chromatography for the Simultaneous Determination of Ketorolac Tromethamine and its Impurities: Multivariate Optimization and Validation. *Journal of Chromatography A*, 1032, 253–263. doi: https://doi.org/10.1016/j.chroma.2003.08.110.

Peng, L.Q., Dong, X., Xiao-Ting, Z., Juan, Y., Yan, Chen., Shu-Ling, W., Tian, Xie., and Jun, C., 2020. Simultaneous Separation and Concentration of Neutral Analytes by Cyclodextrin Assisted Sweeping-Micellar Electrokinetic Chromatography. *Analytica Chimica Acta*, 1105, 224–230. doi: https://doi.org/10.1016/j.aca.2020.01.037.

Rezende, K.C.A., Martins, N.M., Talhavini, M., Wendell, K.T., and Coltro., 2020. Determination of the Alcoholic Content in Whiskeys using Micellar Electrokinetic Chromatography on Microchips. *Food*

Chemistry, 329, 127175. doi: https://doi.org/10.1016/j.foodchem.2020.127175.

Roberto, G., Jessica, F., Sara, B., and Giovanni, D., 2019. Field-amplified Sample Injection and Sweeping Micellar Electrokinetic Chromatography in Analysis of Glyphosate and Aminomethylphosphonic Acid in Wheat. *Journal of Chromatography A*, 1601, 357–364. doi: https://doi.org/10.1016/j.chroma.2019.05.013.

Selvadurai, M., kumar, K.J., and Subramani, P., 2013. Simple and Sensitive Method for the Analysis of Ketorolac in Human Plasma using High-Performance Liquid Chromatography. *Journal of Young Pharmacists*, 5, 98–101. doi: https://doi.org/10.1016/j.jyp.2013.06.007.

Settaluri, V.S., and Division, B., 2015. Validation of Novel and Cost Effective Spectroscopic Methods for Estimation of Ketorolac. *International Journal of Pharmaceutical Sciences and Research*, 6, 4681–4685. doi: https://doi.org/10.13040/IJPSR.0975-8232.6(11).4681-85.

Shigeru, Terabe., Koji, O., Kunimichi, I., Akihiro, T., and Teiichi, A.. 1984. Electrokinetic separations with micellar solutions and open-tubular capillaries. *Analytical Chemistry*, 56, 111–113. doi: https://doi.org/10.1021/ac00265a031.

Silva, M., 2011. Micellar Electrokinetic Chromatography: A Practical Overview of Current Methodological and Instrumental Advances. *Electrophoresis*, 32, 149–165. doi: https://doi.org/10.1002/elps.201000344.

Sunil, G., Jambulingam, M., Ananda, T.S., Kamalakannan, D., Sundaraganapathy, R., and Jothimanivannan, C., 2017. Development and Validation of Ketorolac Tromethamine in Eye Drop Formulation by RP-HPLC Method. *Arabian Journal of Chemistry*, 10, S928–S935. doi: https://doi.org/10.1016/j.arabjc.2012.12.031.

Wan Ibrahim, W.A., Hermawan, D., Sanagi, M.M., Aboul-Enein, H.Y. 2010. Stacking and Sweeping in Cyclodextrin-modified MEKC for Chiral Separation of Hexaconazole, Penconazole and Myclobutanil. *Chromatographia*, 71, 305–309. doi: https://doi.org/10.1365/s10337-009-1427-y.