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Research Article

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A Simple and Validated Spectrophotometric Method for Determination of Piroxicam in Dosage Forms and Biological Fluids

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Abstract A simple and selective spectrophotometric method was developed for the determination Piroxicam (PRX) is proposed and applied to determine the substance in tablets and biological fluids. This method for determination of PRX in aqueous media, which allows quantitation over the $6x10^{-6}$ to $3x10^{-5}$ Mrange for thespectrophotometric methodis proposed. The repeatability and reproducibility of amethod for serum and urine samples are determined. Precision and accuracy are also checked in all media. The standard addition method is used in biological media. The proposed method was checked for determination of PRX in real human serum, urine samples and selectivity of the method over the metabolites was found to be entirely satisfactory.

Keywords piroxicam, spectrophotometry, determination, biological fluids

Introduction

Oxicam is members of a class of non-steroidal anti-inflammatory (NSAIDs) drugs. Piroxicam is a well-known non-steroidal anti-inflammatory and analgesic agent of the oxicam class. Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most frequently used medicinal drugs [1]. They are utilized primarily as analgesics, anti-inflammatories,and antipyretics and their side effects have been well studied. Their main known mode of action is through inhibition of the cyclooxygenase mediated production of prostaglandins. Piroxicam is an NSAID and, as such, is a non-selective COX inhibitor possessing both analgesic and antipyretic properties. It undergoes enterohepatic circulation [2].

Piroxicam shows chemopreventive and chemosuppressive activity in different cancerous cell lines and animal models [3] and also acts as a good photosensitizer induced by UV light [4]. Piroxicam is currently marketed in 20 mg tablets in the name of Felden Flash,and once-daily dosing has proven effective for many patients.

PRX is chemically known as [4-hydroxy-2-methyl-N -(2-pyridyl)-H-1,2-benzothiazine-3-carboxamide-1,1-di-oxide]. The chemical structure has been given in Fig 1.

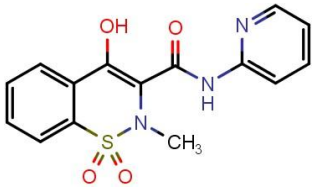

Figure 1: Chemical Structure of PRX

Several methods have been described for the piroxicam determination pharmaceutical formulations and biological fluids. These methods include; spectrophotometry [5–11], chromatography[12,13], voltammetry [14–19] and spectrofluorometry [1,20].

These methods are, either not sufficiently sensitive or tedious and require highly sophisticatedinstrumentation. Furthermore, no analytical methods for the determination of PRX, either in pharmaceutical dosage forms or bulk form and biological fluids, appear to have been reported to date.Several chemical reagents have been used to recommend validation in previous studies [6,14,21–26]. In this study, quantification could be performed without the need for any reagent or chemical agent. More importantly, measurements were taken in the aqueous medium without any buffering during all experiments.

In this study, we have first shown thatdevelopment of aspectrophotometric method for the direct determination of PRX in pharmaceutical dosage forms, spiked human serum and urine without any time-consuming extraction or evaporation steps before drug assay. This work describes fully validated, simple, rapid, selective and sensitive procedures for the determination of PRX employing thespectrophotometric method.

Materials and Methods

Apparatus

Perkin-Elmer Lambda 45 UV-vis double beam spectrophotometer with a slit width of 2 nm. The absorbance values were measured using 1 cm quartz cells.

Reagents

PRX and its dosage forms (Felden Flash) tablets (20 mg) were kindly provided by Pfizer Co. (Istanbul, Turkey). All chemicals were reagent grade (Merck or Sigma). Standard solutions were prepared by dilution of the stock solution containing PRX in the concentration range of $6x10^{-6}$ to $3x10^{-5}$ M. The calibration equation drug analysis was constructed absorbans against PRX concentration.

Preparation of standard and quality control solutions

An aqueous primary stock solution of $1 \cdot 10^{-3}$ M of PRX were prepared in methanol. All measurements were performed at 25 ^{o}C. The standard solutions were prepared by the suitable dilutions of the primary stock solution with ultra-pure water to obtain working standards in the concentration range of $6x10^{-6}$ to $3x10^{-5}$M for PRX. The absorbance's of these solutions werethen fitted in the calibration curve to calculate the accuracy and precision of the method.

Preparation of sample tablet solution

Ten tablets of Valtrex (each tablet contains 20 mg PRX) were accurately weighted and finely powdered by pestle in a mortar. A weighed portion of this powder equivalent to $1x10^{-3}$ M of PRX was transferred into a 100 mL calibrated flask and completed to the volume with bi-distilled water and acetonitril (1:1). The content of the flask were sonicated for 10 min. to effect complete dissolution. Appropriate solution were prepared by taking suitable aliquiots of the clear supernatant liquor and diluting with the selected buffer solutions in order to obtain a final solution.The amount of PRX per tablet was calculated using the linear regression equation obtained from the calibration curve of pure PRX.

Recovery experiment from dosage forms

Recovery of the analytes of interest from a given matrix can be used as a measure of the accuracy or the bias of the method. The same range of concentrations as employed in the linearity studies was used. To study the accuracy, precision and reproducibility of the proposed methods and to check the interference from the excipients used in the dosage forms, recovery experiments were carried out using the standard addition method. These studies were performed by addition of known amounts of pure PRX to the pre-analysed dosage forms and the mixtures were analysed by the proposed techniques. After parallel analyses, the recovery results were calculated using the related calibration equations.

Analysis of spiked serum samples

Serum samples of healthy individuals (after having obtained their written consent) were stored frozen until assay. After gentle, thawing, an aliquot volume of serum sample was spiked with PRX dissolved in bi-distilled water and acetonitrile (1:1) to achieve final stock solution concentration of $1x10^{-3}$ M and treated 5.4 mL of acetonitrile as serum denaturing and precipitating agent, and then the volume was completed to 3.6 mL with the

same serum sample. The samples were vortexed for 10 min and then centrifuged for 5min at 5000 rpm for removing of protein residues. The supernatant of the sample was taken carefully. The concentration of PRX was varied in the range of $6{,}5x10^{-6}$–$1x10^{-5}$M in human serum samples. The amount of PRX in spiked human serum samples for the recovery studies was calculated from the related regression equations. All validation parameters were investigated and calculated for the human serum experiments.

Analysis of spiked urine samples

The drug free urine samples were taken from one healthy female subject immediately before the experiments. An aliquot volume of urine sample was spiked with PRX dissolved in bidistilled water to achieve final concentration of 1×10^{-3} M and treated with 5.4mL acetonitrile as denaturing and precipitating agent for proteins and endogenous substances, and then the volume was completed to 3.6 mL with the same urine sample. The tubes were vortexed for 10 min and then centrifuged for 5 min at 5000 rpm for getting rid of residues. The supernatant was taken carefully. The concentration of PRX was varied in the range of $1x10^{-5}$–$3{,}5x10^{-5}$ M in urine samples. These serial dilutions were analyzed in the deionized water. The amount of PRX in spiked urine samples for the recovery studies was calculated from the related regression equations. Necessary validation parameters were also calculated for urine samples.

Method validation

Linearity: The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method. The calibration curves (y=mx+n) were constructed by the plots of the absorbans (y) of the PRX and the concentrations (x) of the calibration standards. Seven for PRX a point calibration curve was generated with appropriate volumes of working standard solutions for UV methods.

Precision and accuracy: Precision is the degree of repeatability of an analytical method under normal operational conditions. The precision and accuracy were determined with standard quality control samples (in addition to calibration standards) prepared in triplicates at different concentration levels covering the entire linearity range. The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day) and reported as RSD % for a statistically significant number of replicate measurements [27]. The intermediate precision was studied by comparing the assays on three different days and the results are documented as standard deviation and RSD %. Accuracy is the percent of analyte recovered by assay from a known added amount. Data from nine determinations over three concentration levels covering the specified range were obtained. The repeatability of the method was determined by assaying five sample solutions of the highest test concentration [28].

LOD and LOQ: The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from background levels. In this study, LOD and LOQ were based on the standard deviation of response and the slope of the corresponding curve using following equations[29].

$$LOD = 3s/m \;;\; LOQ = 10s/m$$

Where s, the noise estimate, is the standard deviation of the absorbance of the sample, m is the slope of the related calibration graphs. The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with acceptable accuracy, precision and variability[29].

Result and Discussion

The UV spectrophotometer has a common use in drug analysis due to its ability to analyze rapidly with good reproducibility. The key to the application of UV spectrophotometry to drug analysis is to ensure that the matrix components do not affect the active ingredient signal. The solubility of PRX tablets and active ingredient was dissolved using ultrapure water.

The absorption spectra of PRX in aqueous solutions are illustrated in Fig.2-a. Calibration curve data were constructed in the range of expected concentrations of $6x10^{-6}$–$3x10^{-5}$M, $6{,}5x10^{-6}$–$1x10^{-5}$and $1x10^{-5}$–$3{,}5x10^{-5}$for PRX, serum (Fig. 2-b) and urine (Fig. 3-c), respectively. Beer's law was obeyed over this concentration range. The regression equations were found;

$y = 23998x + 0{,}0781$, $R^2 = 0{,}9999$ for PRX

$y = 240229x - 1{,}4082$, $R^2 = 0{,}9966$for serum and

$y = 20730x + 0{,}2133$, $R^2 = 0{,}9992$ for urine.

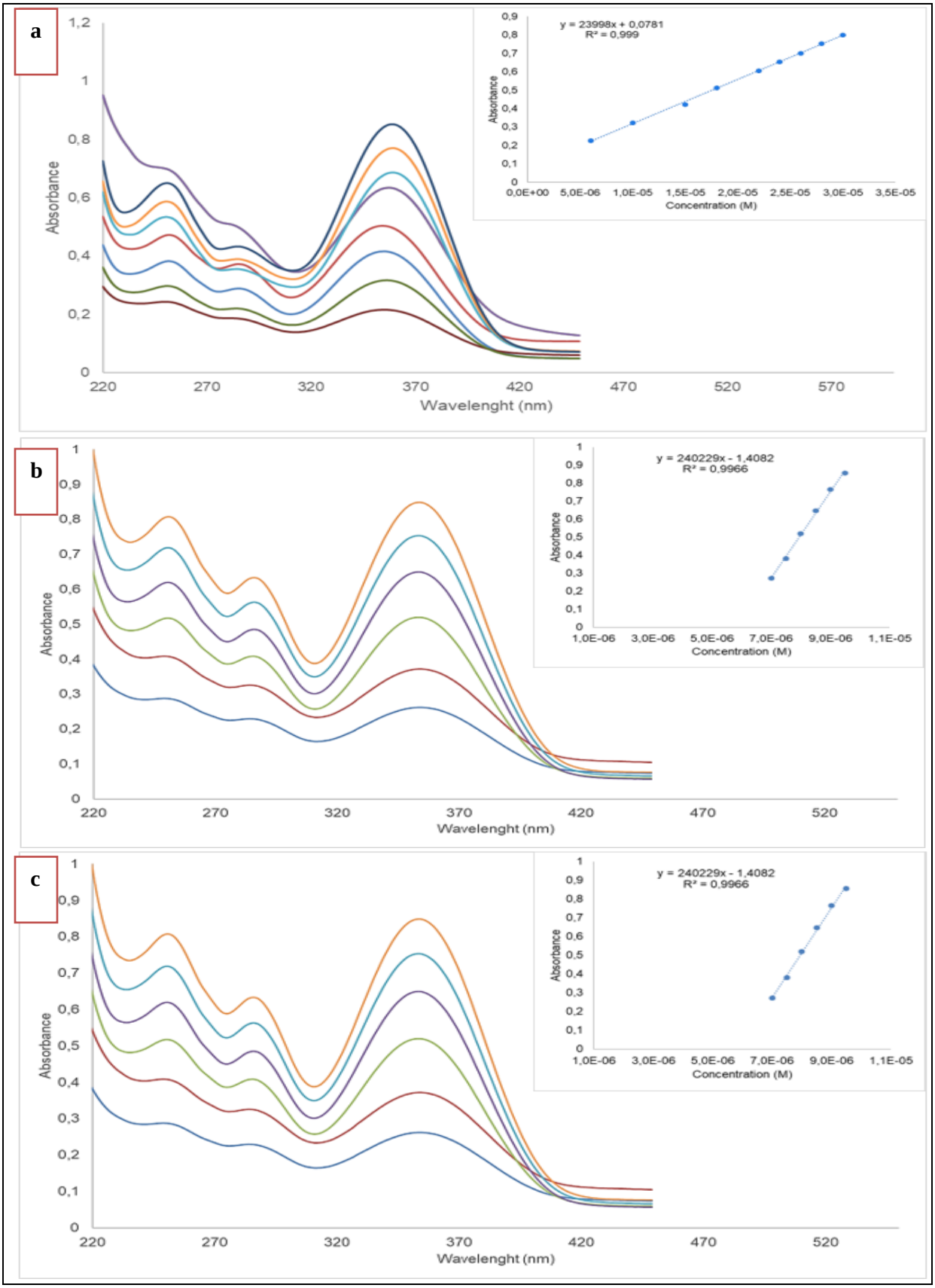

Figure 2: The UV spectra of the active ingredients at various concentrations and the calibration curves obtained from these spectra, PRX(a), Serum (b), Urine (c).

The stock solutions and working standards were made in ultra pure water media. The λ_{max} of the drugs for analysis was determined by taking scans of the drug sample solutions in the entire UV region (355-357 nm).
The characteristics of the calibration plots are summarized in Table 1 and the analytical characteristics and necessary validation parameters for UV techniques for PRX.Performing replicate analyses of the standard solutions was used to assessed the accuracy, precision and reproducibility of the proposed methods. The selected concentration within the calibration range was prepared in water and analyzed with the relevant calibration curves to determine intra-day and inter-day variability. The intra and inter-day precision were determined as the RSD %. The precision, accuracy and reproducibility results given in Table 1 demonstrate a good precision, accuracy and reproducibility.

Table 1: Regression data of the calibration lines for quantitative determination of PRX,serum and urine by the proposed procedure

	Spectrophotometric Method		
	Active Ingredient	Serum	Urine
Measured wavelength (□ max)	355	357	356
Linearity range (mol/L)	6x10^{-6} –3x10^{-5}	6,5x10^{-6} –1x10^{-5}	1x10^{-5} –3,5x10^{-5}
Slope	2,4x10^{4}	2,4x10^{5}	2,0x10^{4}
Intercept	0,0781	-1,4082	0,2133
Correlation coefficient (r)	0.999	0.996	0.999
LOD (mol/L)	2,47x10^{-5}	2,81x10^{-6}	2,63x10^{-5}
LOQ (mol/L)	8,25x10^{-5}	9,37x10^{-6}	8,78x10^{-5}
Repeatability of absorbance (RSD%)	0.300	0.236	0.94
Repeatability of wavelength (RSD%)	0.920	0.286	1.16
Reproducibility of absorbance (RSD%)	0.021	0.003	0.005
Reproducibility of wavelength (RSD%)	0.026	0.009	0.043

The commercial dosage form showed 101.17% recovery by this method, which were within the specified limits of content uniformity. Moreover, the UV method offers a cost effective and time saving alternative to other methods for example colorimetric, complexometric and chromatographic of analysis.
The proposed methods can be successfully applied for PRX assay in dosage forms without any interference. The assay showed the drug content of this product to be in accordance with the labeled claim (Table 2). The recovery of the analyte of interest from a given matrix can be used as a measure of the accuracy of the method. In order to check the accuracy and precision of the developed method and to prove the absence of interferences by excipients, recovery studies were carried out using the standard addition technique. Recovery studies were carried out after the addition of known amounts of the pure drug to various pre-analyzed formulations of all drugs. The application of this procedure is explained in Experimental Section. The obtained results demonstrate the validity and accuracy of the proposed method for the determination of drug in tablets (Table 2). These results reveal that the method has adequate precision and accuracy, and consequently, can be applied to the determination of drug forms in pharmaceuticals without any interference from the excipients.

Table 2: Assay results from PRX dosage forms and mean recoveries in spiked dosage forms

Validation Parameters	**Tablet**
Labeled claim (mg)	20.00
Amount found (mg)[a]	19.67
RSD %	3.3281
Bias %	1.5694
Added (mg)	20.00
Found (mg)[a]	20.23
Recovery %	101.17
RSD % of recovery	3.587
Bias % for Recovery	-1.174

[a] Each value is the mean of 5 experiments

In order to check, the possibility of applying the proposed technique to the human serum and urine samples, the calibration equations were obtained in spiked human serum and urine samples. The determination of PRX in two biological samples could be achieved adopting the UV modes in water as blank solution. Acetonitrile and methanol were tried as the biological sample precipitating agents. Also different amount of acetonitrile and methanol were examined. The best results were obtained using 5.4 mL acetonitrile. The measurements of PRX in human serum and urine samples were performed as described in Experimental section. The applicability of the proposed methods to the human serum and urine samples, the calibration equations were obtained in spiked human serum and urine samples. Calibration equation parameters and necessary validation data were shown in Table 1. Obtained recovery results of spiked human serum and urine samples were given in Table 3. Analysis of drugs from biological samples such as human serum and urine samples usually requires extensive time-consuming sample preparation, use of organic solvents and other chemicals. In this study, the serum or and urine proteins and endogenous substances in serum and urine samples as precipitated by the addition of acetonitrile, which is centrifuged at 5000xg and the supernatant was taken and diluted with the selected buffer solution and directly analyzed. Using proposed technique, no sample pretreatment was required, other than precipitation and dilution steps. The recovery results of PRX (Table 3) in human serum and urine samples were calculated from the related linear regression equations, which are given in Table 1. Stability of the serum and urine samples kept in refrigerator (+4 ^{o}C) was tested by making five consecutive analyses of the sample over a period of approximately 5h. There were no significant changes in the peak absorbances and wavelengths between the first and last measurements.

Table 3: Application of the spectrophotometric procedure to the determination of PRX in spiked human serum and urine medium

Medium	PRX added (mg)	n	PRX Found (mg)	Average recovery %	RSD %	Bias %
Serum	2.319	8	2.233	96.29	0.387	3.71
Urine	3.976	8	3.923	98.66	7.516	1.34

Conclusion

The presented study suggests a simple, inexpensive, precise and accurate method for PRX detection in pharmaceutical preparations and biological fluids. The proposed methods are advantageous over the spectrophotometric, colorimetric, voltammetric and chromatographic methods mentioned in the introduction section. The most striking advantage of spectrophotometric methods is the sensitivity that exceeds the sensitivity of some of the previously reported methods. A significant advantage of the spectrophotometric method is that it can be applied to the identification of individual compounds in a multicomponent mixture. Moreover, in this method, harmful chemicals used in other spectrophotometric methods were not used and ultrapure water was used as a rubbish. In summary, the proposed method can be used for drug analysis in routine quality control.

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References

[1]. Damiani, P. C., Bearzotti, M., Cabezón, M., and Olivieri, A. C., Spectrofluorometric determination of piroxicam, *Journal of Pharmaceutical and Biomedical Analysis*, 17: (2), 233–236 (1998).

[2]. Ding, H., Han, C., Gibson-D'Ambrosio, R., Steele, V. E., and D'Ambrosio, S. M., Piroxicam selectively inhibits the growth of premalignant and malignant human oral cell lines by limiting their progression through the S phase and reducing the levels of cyclins and AP-1, *International Journal of Cancer*, 107: (5), 830–836 (2003).

[3]. Üstün Alkan, F., Üstüner, O., Bakırel, T., Çınar, S., Erten, G., et al., The Effects of Piroxicam and Deracoxib on Canine Mammary Tumour Cell Line, *The Scientific World Journal*, 2012:, 1–8 (2012).

[4]. Banerjee, R., Chakraborty, H., and Sarkar, M., Photophysical studies of oxicam group of NSAIDs:

piroxicam, meloxicam and tenoxicam, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 59: (6), 1213–1222 (2003).

[5]. Dhage, M. A., Chhabra, G. S., and Banerjee, S. K., Development and validation of UV-spectrophotometric method for piroxicam in bulk and pharmaceutical formulation, *Journal of Chemical and Pharmaceutical Research*, 3: (2), 765–769 (2011).

[6]. Rele, R. V., Sawant, S. A., and Warkar, C. B., Simple spectrophotometric methods for determination of piroxicam in pharmaceutical formulation, *International Journal of ChemTech Research*, 2: (4), 2173–2176 (2010).

[7]. Basan, H., Göer, N. G., Ertaş, N., and Orbey, M. T., Quantitative determination of piroxicam in a new formulation (piroxicam-β-cyclodextrin) by derivative UV spectrophotometric method and HPLC, *Journal of Pharmaceutical and Biomedical Analysis*, 26: (2), 171–178 (2001).

[8]. Ch, O. N., Kormosh, Z. a., Hunka, I. P., and Bazel, Y. R., Spectrophotometric determination of piroxicam, *Journal of Analytical Chemistry*, 66: (4), 378–383 (2011).

[9]. Nagaralli, B. S., Seetharamappa, J., and Melwanki, M. B., Sensitive spectrophotometric methods for the determination of amoxycillin, ciprofloxacin and piroxicam in pure and pharmaceutical formulations, *Journal of Pharmaceutical and Biomedical Analysis*, 29: (5), 859–864 (2002).

[10]. Amin, A. S., Spectrophotometric determination of piroxicam and tenoxicam in pharmaceutical formulations using alizarin, *Journal of Pharmaceutical and Biomedical Analysis*, 29: (4), 729–736 (2002).

[11]. El Didamony, A. M., and Amin, A. S., Adaptation of a Color Reaction for Spectrophotometric Determination of Diclofenac Sodium and Piroxicam in Pure Form and in Pharmaceutical Formulations, *Analytical Letters*, 37: (6), 1151–1162 (2004).

[12]. Puthli, S. P., and Vavia, P. R., Stability indicating HPTLC determination of piroxicam, *Journal of Pharmaceutical and Biomedical Analysis*, 22: (4), 673–677 (2000).

[13]. de Jager, A. ., Ellis, H., Hundt, H. K. ., Swart, K. ., and Hundt, A. ., High-performance liquid chromatographic determination with amperometric detection of piroxicam in human plasma and tissues, *Journal of Chromatography B: Biomedical Sciences and Applications*, 729: (1–2), 183–189 (1999).

[14]. Shahrokhian, S., Jokar, E., and Ghalkhani, M., Electrochemical determination of piroxicam on the surface of pyrolytic graphite electrode modified with a film of carbon nanoparticle-chitosan, *Microchimica Acta*, 170: (1–2), 141–146 (2010).

[15]. Abo El-Maali, N., Hassan, R. M. R., El-Maali, N. A., Hassan, R. M. R., Abo El-Maali, N., et al., Electrooxidation and determination of the anti-inflammatory drugs Piroxicam and Tenoxicam at the carbon paste electrode, *Journal of Electroanalytical Chemistry*, 299: (2), 155–163 (1990).

[16]. Reguera, C., Ortiz, M. C. C., and Arcos, M. J. J., Differential Pulse Voltammetric Simultaneous Determination of Four Anti-Inflammatory Drugs by Using Soft Modelling, *Electroanalysis*, 14: (24), 1699–1706 (2002).

[17]. Paniagua, A. A. R., Vazquez, M. D., Tascon, M. L., Sanchez-Ba Ta N Ero, P., and Vázquez, M., Voltammetric Determination of Piroxicam after Incorporation within Carbon Pastes, *Electroanalysis*, 6:, 265–268 (1994).

[18]. Acuña, J. A., de la Fuente, C., Vãzquez, M. D., Tascón, M. L., Sánchez-Batanero, P., et al., Voltammetric determination of piroxicam in micellar media by using conventional and surfactant chemically modified carbon paste electrodes, *Talanta*, 40: (11), 1637–1642 (1993).

[19]. Beltagi, A. M., Abdallah, O. M., and Ghoneim, M. M., Determination of piroxicam in pharmaceutical formulations and human serum by square-wave stripping voltammetry, *Chemia analityczna*, 387: (52), 2007 (2007).

[20]. Tabrizi, A. B., and Tutunchi, N. S., Analysis of piroxicam in pharmaceutical formulation and human urine by dispersive liquid-liquid microextraction combined with spectrophotometry, *Advanced Pharmaceutical Bulletin*, 3: (1), 37–44 (2013).

[21]. Pascual-Reguera, M. I., Ayora-Cañada, M. J., and Castro Ruiz, M. S., Determination of piroxicam by solid-phase spectrophotometry in a continuous flow system, *European Journal of Pharmaceutical*

Sciences, 15: (2), 179–183 (2002).

[22]. Alizadeh, N., and Keyhanian, F., Sensitive and selective spectrophotometric assay of piroxicam in pure form, capsule and human blood serum samples via ion-pair complex formation, *Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy*, 130:, 238–244 (2014).

[23]. Dragomiroiu, G. T. A. B., Cimpoieşu, A., Ginghină, O., Baloescu, C., Bârcă, M., et al., The development and validation of a rapid HPLC method for determination of piroxicam, *Farmacia*, 63: (1), 123–131 (2015).

[24]. Kormosh, Z., Hunka, I., Bazel, Y., and Matviychuk, O., Potentiometric determination of ketoprofen and piroxicam at a new PVC electrode based on ion associates of Rhodamine 6G, *Materials Science and Engineering: C*, 30: (7), 997–1002 (2010).

[25]. Basan, H., Göer, N. G., Ertaş, N., Orbey, M. T. T., Göğer, N. G., et al., Quantitative determination of piroxicam in a new formulation (piroxicam-β-cyclodextrin) by derivative UV spectrophotometric method and HPLC, *Journal of Pharmaceutical and Biomedical Analysis*, 26: (2), 171–178 (2001).

[26]. Azmi, S., and Iqbal, B., Spectrophotometric Determination of Piroxicam via Chelation with Fe (III) in Commercial Dosage Forms, *Journal of the Chinese Chemical Society*, (56), 1083–1091 (2009).

[27]. Gölcü, A., and Serin, S., Spectrophotometric determination of nifedipine via charge-transfer complexes, *Scientia Pharmaceutica*, 66: (4), 341–349 (1998).

[28]. Golcu, A., Dogan, B., and Ozkan, S. A., Anodic voltammetric behavior and determination of cefixime in pharmaceutical dosage forms and biological fluids, *Talanta*, 67: (4), 703–712 (2005).

[29]. Gölcü, A., New, simple, and validated UV-spectrophotometric method for the estimation of some beta blockers in bulk and formulations, *Journal of Analytical Chemistry*, 63: (6), 538–543 (2008).