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Spectrophotometric Estimation Methods for Loxoprofen Sodium Based on Charge Transfer Complexation Reactions

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ABSTRACT

This work describes the development and validation of five fast, simple, accurate and precise spectrophotometric methods for the determination of loxoprofen sodium (LXP) through charge transfer complexation reactions. The first method is based on the development of a purple colored product with maximum absorbance at 523 nm after reaction of LXP with p-chloranilic acid (p-CA) in acetone. The second method depends on the formation of a bluish-green product measured at 844 nm through the reaction of LXP with 7,7,8,8-tetracyanoquinodimethane (TCNQ) in acetone. The third method involves the formation of a yellow chromogen with a strong absorption maximum at 458 nm upon the reaction of LXP with 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ) in acetonitrile. The fourth method is based upon the production of a yellow complex measured at 414 nm the interaction of LXP and picric acid (PA) in chloroform. The fifth method yields a yellow complex with maximum absorption at 361 nm upon the interaction of LXP with iodine in chloroform. Optimization of experimental conditions that affect the color development was accomplished. Stoichiometry of the reactions was studied. Validation of the proposed spectrophotometric methods was made in terms of linearity, ranges, precision, accuracy, robustness, detection and quantification limits. The formed color products of LXP with p-CA, TCNQ, DDQ, PA and iodine showed good linear relationships over the concentration ranges 40–240, 1–10, 16–96, 8–32 and 2–12 µg/mL respectively. The proposed methods were suitably employed for the assay of LXP in bulk drug and pharmaceutical dosage forms. No spectral interferences from the tablet excipients were found.

Keywords: Loxoprofen sodium ; Spectrophotometric determination ; Charge transfer complex ; p-Chloranilic acid ; TCNQ ; DDQ ; Picric acid ; Iodine.

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INTRODUCTION

Loxoprofen sodium (LXP) is a recently developed novel NSAID (non-steroidal antinflammatory drug) and a prodrug of a propionic acid derivative. It is chemically sodium-2-[4-(2-oxocyclopentyl-1-methyl) phenyl]propionate dihydrate (Fig.1) [1]. It has anti-inflammatory and antipyretic properties.LXP acts by inhibiting isoforms of cyclo-oxygenase 1 and 2. After oral administration, LXP is absorbed as the free acid rather than the sodium salt from the gastrointestinal tract, which causes only weak irritation of the gastric mucosa. Subsequently, it is converted to an active metabolite by reduction of the ketone carbonyl to the trans-OH form. Therefore, LXP is thought to have a relatively weak gastrointestinal ulcerogenicity compared with ibuprofen [2]. LXP is administered orally, also a transdermal preparation was approved for sale in Japan on January 2006 [3]. Recently, NSAIDs have gained greater clinical attention since many research found that they can minimize the risk of developing Alzheimer's disease and various tumors [4-6].

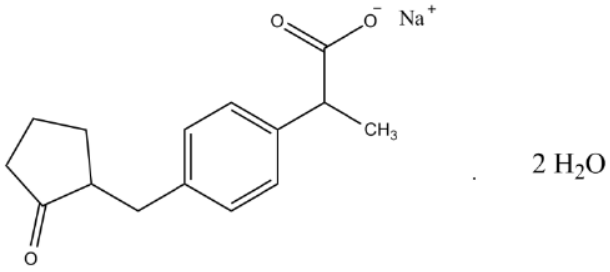

Figure 1: Chemical structure of Loxoprofen Sodium (LXP).

Several studies for the estimation of the LXP using a variety of techniques have been reported, the vast majority of them used HPLC technique. LXP was analyzed simultaneously with its diastereometric alcohol metabolites by using on-line column switching LC [7], HPLC using fluorescence labelling with 4-bromomethyl-6,7-methylenedioxycoumarin [8] or by a simple HPLC-UV detection method [9]. Stereo specific analysis of LXP was carried out using chiral LC [10] and liquid chromatography–tandem mass spectrometry was reported for analysis of loxoprofen in human plasma [11]. The drug was also determined in tablets by simple RP-HPLC with UV detection [12] and in human tears and plasma samples using ultra-fast liquid chromatography-tandem mass spectrometry [13]. In addition, a stability indicating study has been carried out using HPLC and HPTLC techniques together with structure elucidation of the degradation products [14]. Also, identification of degradation products in LXP adhesive tapes was applied using HPLC-MS [15]. On the other hand, gas chromatography-mass spectrometry technique was applied for the simultaneous determination of the drug with other NSAIDs in river water [16]. A capillary zone electrophoresis and micellar electrokinetic chromatography were used for the determination of the drug in human specimens [17]. The drug was also determined in pharmaceutical formulations using flow injection chemiluminescence technique [18].

To the best of our knowledge, no reports have applied any spectrophotometric technique for the determination of LXP. The fact that up till now the colorimetric determination specially the charge transfer complexation reactions of LXP have not been tackled yet since no analytical reports are found in the literature, encouraged us to develop simple, rapid, accurate and reliable spectrophotometric methods for the analysis of LXP for quality control purposes.

Such simple, fast direct and low-cost methods of analysis are very efficient especially for drugs that lack chromophores, and consequently, suffer from shortage of spectrophotometric analytical methods. The establishment of simple, rapid, and adequately sensitive spectrophotometric methods to be applicable for routine analysis in quality control laboratories has been one of the main targets for analytical chemists. Being a salt of weak acid and a negative charge carrier, LXP is a good electron donor “electron rich molecule” and can form charge transfer complexes with various acceptors “electron deficient molecule”. These molecular interactions between electron donors and acceptors usually resulted into the formation of intensely colored charge-transfer complexes, which absorb radiation in the visible region. The same principle was applied with other similar weak acid salts such as sodium valproate, losartan potassium and rabeprazole sodium which

reacted similarly with several electron acceptors, and the produced colored complexes were utilized for their spectrophotometric assays [19-21].

EXPERIMENTAL

Instrumentation

Spectrophotometric measurements were carried out on a T80 double beam UV/VIS spectrophotometer (PG instruments Ltd., London, UK) connected to a PC loaded with UV WIN 5 software (version 5.2.0) using a pair of 1 cm matched quartz cells.

Materials and reagents

Loxoprofen sodium (LXP) was kindly provided by Pharco Pharmaceuticals Co. (Alexandria, Egypt). Analytical grade of 2,5-dichloro-3,6-dihydroxy-1,4-benzoquinone (p-chloranilic acid, p-CA) (BDH Chemicals, Poole, UK), 7,7,8,8-Tetracyanoquinodimethane (TCNQ) (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), 2,3-Dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), 2,4,6-trinitrophenol (picric acid, PA) (S.D. Fine Chem Ltd., Mumbai, India) and iodine (Riedel-de Haën AG, Seelze, Germany) were used. All solvents and other chemicals used throughout this study were of analytical grade. The pharmaceutical preparations used in the present investigation were Roxonin® 60 tablets (SAJA pharmaceuticals, Jeddah, Saudi Arabia under license from Daiichi sankyo company, Tokyo, Japan, B.N. P14M500) labeled to contain 68.1 mg LXP per tablet, Roxogesic® tablets (TAG PHARMA, Cairo, Egypt, B.N. 151391) labeled to contain 68 mg LXP per tablet and Loujain® capsules (HOCHSTER pharmaceutical industries, Bader industrial city, Egypt, B.N. 16C081) labeled to contain 68.1 mg LXP per capsule.

Preparation of LXP stock standard and reagents' solutions

Stock standard solutions of LXP, 2000 µg/mL, 100 µg/mL, 800 µg/mL, 200 µg/mL and 100 µg/mL, were separately prepared in methanol to be used for the reactions with p-CA, TCNQ, DDQ, PA and iodine respectively. Reagents' solutions were freshly prepared in the following concentrations: p-CA (3 mg/mL) in acetone, TCNQ (3 mg/mL) in acetonitrile, DDQ (2 mg/mL) in acetonitrile, PA (0.5 mg/mL) in chloroform and iodine (5 mg/mL) in chloroform.

General procedures and calibration graphs

Method I: volumes (200–1200 µL) of LXP stock standard solution (2000 µg/mL) were transferred into a series of 10-mL volumetric flasks. Adjustment to 2 mL with methanol and addition 0.6 mL of p-CA solution was performed. The volume was adjusted to 10 mL with acetone and the absorbance was measured at 523 nm against reagent blank.

Method II: volumes (100–1000 µL) of LXP stock standard solution (100 µg/mL) were transferred into a series of 10-mL volumetric flasks. Adjustment to 1 mL with methanol and addition of 1.6 mL of TCNQ solution was performed. Solutions were allowed to stand for 10 min, then the volume was adjusted to 10 mL with acetone and the absorbance was measured at 844 nm against reagent blank.

Method III: volumes (200–1200 µL) of LXP stock standard solution (800 µg/mL) were transferred into a series of 10-mL volumetric flasks. Adjustment to 1.5 mL with methanol and addition of 0.8 mL of DDQ solution was performed. Solutions were left for 10 min at room temperature, then the volume was adjusted to 10 mL with ACN and the absorbance was measured at 458 nm against reagent blank.

Method IV: volumes (400–1600 µL) of LXP stock standard solution (200 µg/mL) were transferred into a series of 10-mL volumetric flasks. Adjustment to 2 mL with methanol and addition of 0.6 mL of PA solution was performed. The volume was adjusted to 10 mL with chloroform and the absorbance was measured at 414 nm against reagent blank.

Method V: volumes (200–1200 µL) of LXP stock standard solution (100 µg/mL) were transferred into a series of 10-mL volumetric flasks. Adjustment to 1.5 mL with methanol and addition of 0.8 mL of iodine

solution was performed. Solutions were left at room temperature for 10 min, then volumes were completed to 10 mL with chloroform and the absorbance was measured at 361 nm against reagent blank.

For production of calibration graphs, absorbance values for each method were plotted against the corresponding drug concentrations.

Assay of LXP dosage forms

For both tablet formulations, ten tablets were weighed and finely powdered. On the other hand, the content of ten capsules was accurately weighed. For each dosage form, weights of the powder equivalent to 200, 80 and 20 mg LXP were extracted into separate volumes of 50 mL methanol by sonication for 30 min then filtered into separate 100 mL-volumetric flasks. The residues were washed with methanol and washings were added to the filtrates, volumes were completed to 100 mL with methanol to reach final concentrations 2000, 800 and 200 µg/mL LXP (stock sample solutions for reactions with p-CA, DDQ and PA, respectively). For the reaction with TCNQ and iodine, weight of the powder equivalent to 10 mg LXP was similarly treated to reach a final concentration 100 µg/mL LXP (stock sample solution for reaction with TCNQ and iodine). Volumes of the prepared stock sample solutions were transferred into 10 mL volumetric flasks, and the assays were conducted as mentioned under general procedures. Calculation of recovery values from similarly treated standard solutions was done.

RESULTS AND DISCUSSION

Spectral characteristics and proposed mechanisms of the reactions

Charge-transfer complexation is usually performed between an electron donor and an electron acceptor, accompanied by electronic transition(s) to an excited state and a partial transfer of electronic charge from the donor to the acceptor. As a conclusion, the excitation energy of this resonance occurs usually in the visible region of the electromagnetic spectrum.

Since loxoprofen carboxylate anion exists in the solvent as an ionized negatively charged species, it acts as a powerful electron donor and reacts immediately with p-CA (as π-acceptor), yielding a purple colored product peaking at 523 nm (Figure 2). Also, LXP reacts with TCNQ to produce a bluish-green product possessing major absorption bands at 844, 824, 762 and 744 nm where the 844 nm is selected for further validation since it possesses the maximum absorption intensity (Figure 3). Likewise, LXP reacts with DDQ resulting in the production of an orange yellow color, which shows three maxima at 582, 543 and 458 nm. The band at 458 nm, is chosen for construction of Beer's plot since it shows the highest absorption intensity (Figure 4). As well, LXP yields a yellow product with an absorption maximum at 414 nm after reaction with PA (Figure 5). The formation of these colored products can be attributed to charge-transfer complexes production, pursued by dissociation of the donor-acceptor complexes yielding colored radical anions of p-CA, TCNQ, DDQ and PA. The aforementioned reaction scan be explained by the following scheme:

$$\underset{\text{Donor}}{D} + \underset{\pi\text{-acceptor}}{A} \rightleftharpoons \underset{\text{Complex}}{D{-}A} \rightleftharpoons \underset{\text{Radical ions}}{D^{\bullet+} + A^{\bullet-}}$$

Furthermore, various published reports indicate that the reactions of electron rich compounds with PA occur through intermolecular hydrogen bonding [22-24]. This consistently happens between the basic group on LXP (the carboxylate anion) and the acidic group on PA (the phenolic group).

$$RCOO^{-} \cdots H{-}O{-}Ar \rightleftharpoons RCOO{-}H \cdots {}^{-}O{-}Ar$$

$$D + I_2 \rightleftharpoons \underset{\text{Outer complex}}{D{-}I_2} \overset{I_2}{\rightleftharpoons} \underset{\text{Inner complex}}{[D{-}I]^{+}\, I^{-}} \rightleftharpoons [D{-}I]^{+}\, I_3^{-}$$

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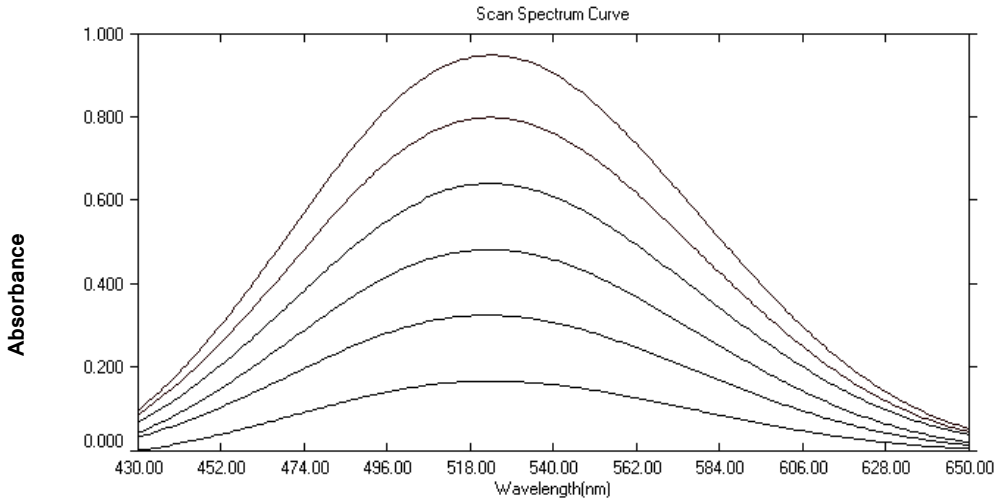

Figure 2: Absorption spectra of the reaction product of different concentrations of LXP (40, 80, 120, 160, 200 and 240 µg/mL) with p-CA in acetone.

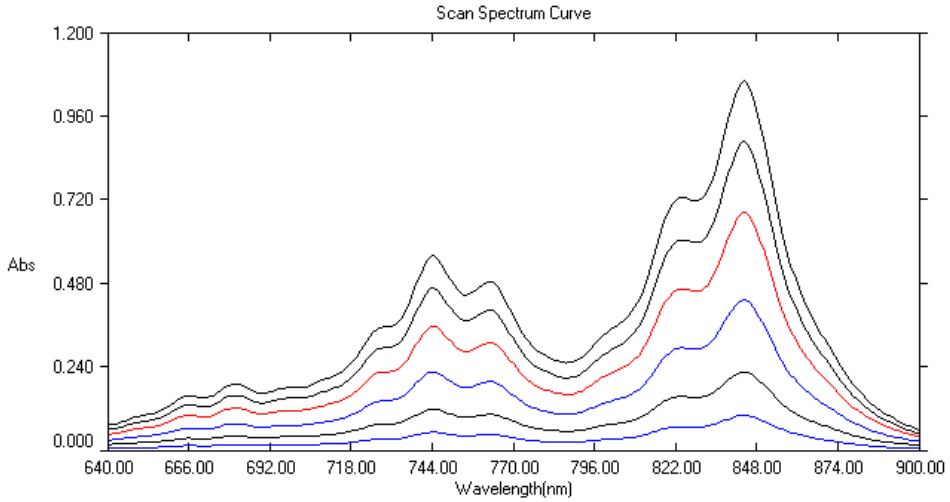

Figure 3: Absorption spectra of the reaction product of different concentrations of LXP (1, 2, 4, 6, 8 and 10 µg/mL) with TCNQ in acetone after 10 min.

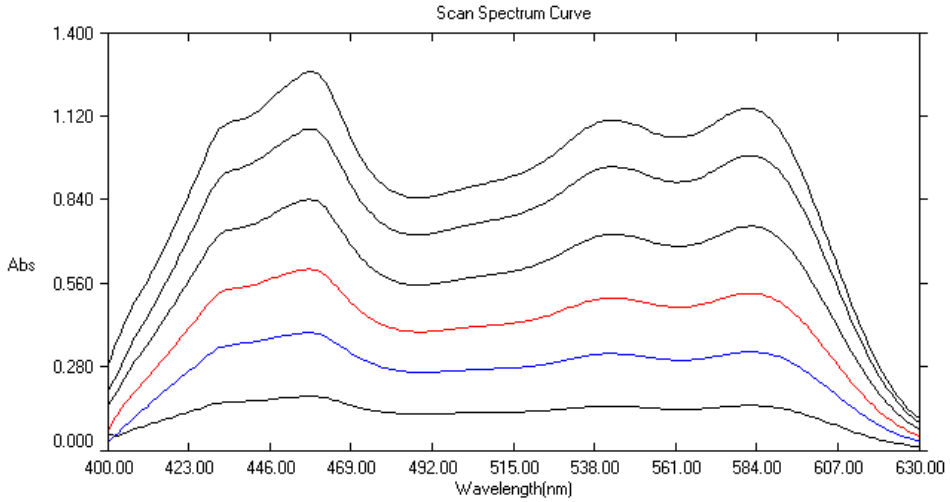

Figure 4: Absorption spectra of the reaction product of different concentrations of LXP (16, 32, 48, 64, 80 and 96 µg/mL) with DDQ in acetonitrile after 10 min.

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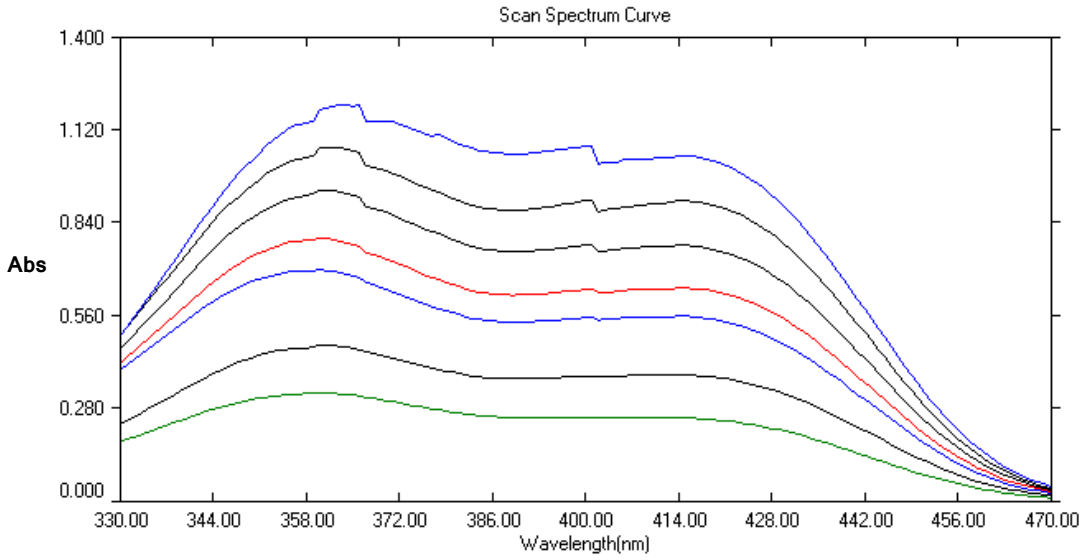

Figure 5: Absorption spectra of the reaction product of different concentrations of LXP (8, 12, 16, 20, 24, 28 and 32 µg/mL) with PA in chloroform.

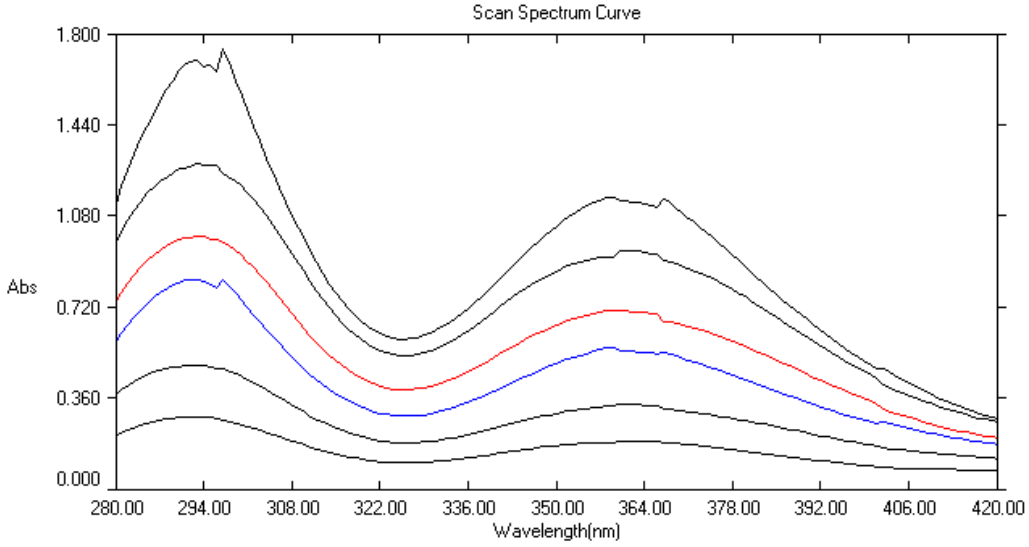

Figure 6: Absorption spectra of the reaction product of different concentrations of LXP (2, 4, 6, 8, 10 and 12 µg/mL) with iodine in chloroform after 10 min.

In contrast, the formation of a charge transfer complex after reaction with iodine considering LXP as an electron donor and iodine as σ acceptor, and the absorption spectrum of LXP-iodine reaction product possessed absorption peaks at 293 and 361nm (Figure 6). A possible explanation of the reaction is the formation of an outer complex that dissociates into an inner complex yielding iodide (I^-) that reacts with the free molecular iodine to yield the tri-iodide species (I_3^-) that possess the distinguished absorption spectrum with the two peaks at 293 and 361 nm.

The relative sensitivity of the five acceptors employed in the present analytical work may be attributed to their difference in electron affinities, as well as the conditions employed in the reactions (reagent concentration, reaction time, and solvent polarity). The relative sensitivity of the acceptors may be compared by their ε values. TCNQ exhibits the highest ε value related to its high electron affinity. The weak and small value in case of p-CA may be explained on the basis of insufficient ionization of this relatively weak β-acceptor which possesses lower electron affinity.

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Optimization of experimental conditions

Diverse parameters influencing the development of the colored products and their stability were cautiously tested and optimized. Such parameters were changed individually while maintaining the others constant. The effect of the concentration of the reagent (in terms of reagent volume) on the absorbance intensity was tested. It was found that 0.6 mL of 3 mg/mL p-CA solution, 1.6 mL of 3 mg/mL TCNQ solution, 0.8 mL of 2 mg/mL DDQ solution, 0.6 mL of 0.5 mg/mL PA solution and 0.8 mL of 5 mg/mL iodine solution were adequate for maximum and reproducible color intensity production (Figure 7).

With the purpose of selecting the most suitable diluting solvent, the reactions were conducted in different organic solvents such as acetone, acetonitrile, ethanol, methanol, DMF, methylene chloride and chloroform. Tiny shifts in the location of the maximum absorption peak were noticed, while the absorption intensities were widely affected. In most cases, both alcohols and DMF were ignored because they yielded low absorption readings. Acetone was favored to acetonitrile because of the higher color intensity of the p-CA and TCNQ complexes formed in it. Furthermore, acetonitrile increases the maximum sensitivity in comparison to other solvents upon reaction with DDQ. Regarding the reactions with PA and iodine, chloroform was better than other solvents, therefore, it was selected as an ideal solvent for these reactions.

The most suitable reaction time was tested by monitoring the color development at room temperature (20 ± 2 °C). Full color development was reached immediately with p-CA and PA, and increasing time of the reactions did not result into any increase in the absorbance. Accordingly, the absorbance of product was measured at zero time. In case of TCNQ, DDQ and iodine, highest color intensity was attained after 10 min; therefore, the reactions with these reagents were allowed to stand for 10 min before recording the absorbance. The produced colors were stable for at least a further 30 min.

The effect of temperature on the spectrophotometric response was studied in case of DDQ and TCNQ. It was found that heating has no effect on reaction response with TCNQ while absorbance of the formed DDQ charge transfer complex decreased gradually by rising the temperature. Thus, these reactions were carried out at room temperature.

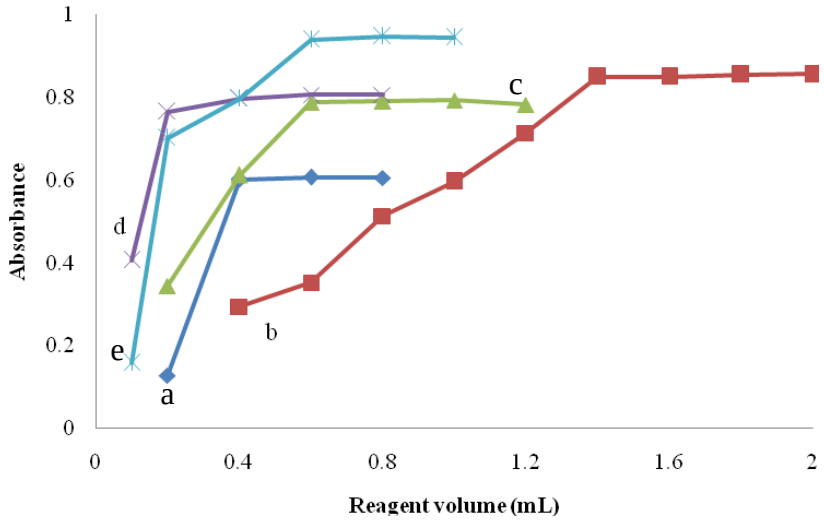

Figure 7: Effect of reagent volume on the absorbance of the reaction product of (a) 150 µg/mL LXP with p-CA, (b) 8 µg/mL LXP with TCNQ, (c) 60 µg/mL LXP with DDQ, (d) 25 µg/mL LXP with PA and (e) 10 µg/mL LXP with iodine.

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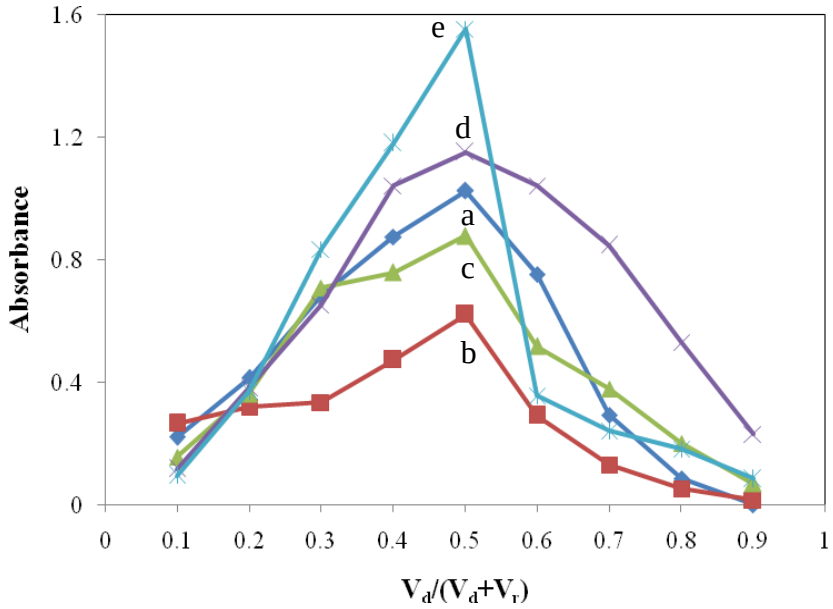

Figure 8: Continuous variation plots for the reactions of LXP with (a) p-CA, (b) TCNQ, (c) DDQ, (d) PA and (e) iodine.

Stoichiometry of the reactions:

Job's method of continuous variation was applied to study the stoichiometry of the reactions [25] using equimolar concentrations of the drug and reagents (16×10^{-3} M and 2×10^{-3} M for reactions with p-CA and PA, respectively and 5×10^{-3} M for reactions with TCNQ, DDQ and iodine). Job's plot reached a highest value at a mole fraction of 0.5 demonstrating a molar reaction ratio of 1:1 for the drug (LXP) with all acceptors studied (Figure 8). This reveals that only one center is responsible for the complex formation.

Validation of the proposed methods:

Validation of the proposed spectrophotometric methods was carried out according to the International Conference on Harmonization (ICH) guidelines on validation of analytical procedures [26].

Linearity and concentration ranges

Linear relationships occur between the absorbance of the color products and the corresponding concentrations of LXP using the optimized reactions conditions. Table 1 demonstrate the linearity data and statistical parameters for the proposed methods including linear regression equations, concentration ranges, correlation coefficients, molar absorptivity values (ε), standard deviations of the intercept (S_a), the slope (S_b) and standard deviations of residuals ($S_{y/x}$). In order to indicate the random error in the estimated values of "y", an important statistical criterion is calculated that is the standard deviation of residuals, $S_{y/x}$. The smaller its value the closer the points are to the straight line. Regression analysis reveals good linearity as declared from the small intercepts, good correlation coefficient values ($r > 0.9994$) and RSD% of the slope values which were found less than 2 %.

Limits of detection and quantification

The LOD and LOQ values were calculated and showed in Table 1. Clearly, the LOD, LOQ and apparent molar absorptivity values highlight that the reactions with TCNQ and iodine offer greater sensitivity of measurement of LXP through the proposed methods.

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Table 1: Analytical parameters for the determination of LXP using the proposed charge transfer spectrophotometric methods.

Parameter	p-CA	TCNQ	DDQ	PA	Iodine
Wavelength (nm)	523	844	458	414	361
Linearity range (µg/mL)	40 - 240	1 - 10	16 - 96	8 - 32	2 - 12
Apparent molar absorptivity (ε) ($L\ mol^{-1}\ cm^{-1}$)	1187	31678	4230	10042	30460
Intercept (a)	0.0117	0.0075	-0.0485	- 0.0156	-0.0533
Slope (b)	0.0039	0.1041	0.0139	0.0330	0.1001
Correlation coefficient (r)	0.99986	0.99949	0.99973	0.99993	0.99992
S_a	0.0051	0.0101	0.0099	0.0038	0.0049
S_b	3.27×10^{-5}	0.0017	1.66×10^{-4}	1.76×10^{-4}	6.27×10^{-4}
RSD% of slope (S_b)	0.84	1.63	1.19	0.53	0.63
$S_{y/x}$	0.0055	0.0130	0.0106	0.0037	0.0052
LOD (µg/mL)	4.32	0.32	2.35	0.38	0.16
LOQ (µg/mL)	13.08	0.97	7.12	1.15	0.49

Accuracy and precision

Study of the accuracy and within-day precision (repeatability) for the proposed methods were performed at three concentration levels of LXP using three replicate determinations for each concentration within one day. In the same way, study of the accuracy and between-day precision (intermediate precision) were accomplished through analysis of the same three concentrations using three replicate determinations repeated on three days. Calculation of the recovered concentrations was made using the corresponding regression equations and were satisfactory. Summary of the analytical results get from this investigation is presented in Table 2. The high precision and good accuracy of the proposed methods for the determination of LXP in bulk form were indicated by the low values of percentage relative standard deviation (RSD %) and percentage relative error (E_r %) (Not more than 2%).

Table 2: Accuracy and precision for the analysis of LXP in bulk form using the proposed spectrophotometric methods.

Reagent	Type of analysis	Nominal value (µg/mL)	Found ± SD[a] (µg/mL)	RSD(%)[b]	E_r(%)[c]
p-CA	Intra-Day	80	79.72 ± 0.67	0.84	-0.35
		160	160.28 ± 0.73	0.46	0.18
		240	238.99 ± 0.67	0.28	-0.42
	Inter-Day	80	79.72 ± 1.11	1.39	-0.35
		160	161.97 ± 2.30	1.42	1.23
		240	241.27 ± 3.30	1.37	0.53
TCNQ	Intra-Day	2	2.02 ± 0.029	1.44	1.00
		6	5.93 ± 0.053	0.89	-1.17
		10	10.04 ± 0.092	0.92	0.40
	Inter-Day	2	2.02 ± 0.034	1.68	1.00
		6	6.01 ± 0.095	1.58	0.17
		10	10.03 ± 0.111	1.11	0.30
DDQ	Intra-Day	32	31.83 ± 0.36	1.13	-0.53
		64	64.48 ± 0.40	0.62	0.75
		96	95.31 ± 0.29	0.30	-0.72
	Inter-Day	32	31.45 ± 0.57	1.81	-1.72
		64	64.58 ± 0.69	1.07	0.91
		96	95.72 ± 0.40	0.42	-0.29

PA	**Intra-Day**	12	11.97 ± 0.06	0.50	-0.25
		20	20.13 ± 0.13	0.65	0.65
		28	27.93 ± 0.09	0.32	-0.25
	Inter-Day	12	12.02 ± 0.17	1.41	0.17
		20	20.05 ± 0.19	0.95	0.25
		28	28.00 ± 0.29	1.04	0.00
Iodine	**Intra-Day**	4	3.98 ± 0.05	1.26	-0.50
		8	7.93 ± 0.07	0.88	-0.88
		12	11.84 ± 0.10	0.84	-1.33
	Inter-Day	4	3.99 ± 0.06	1.50	-0.25
		8	7.93 ± 0.08	1.01	-0.88
		12	11.85 ± 0.15	1.27	-1.25

[a] Mean ± standard deviation for three determinations. [b] % Relative standard deviation. [c] % Relative error.

Robustness

Robustness was tested by making small changes in the working wavelength (± 3 nm), reagent volume (± 0.2 mL for TCNQ and ± 0.1 mL for other reagents) and time in case of reaction with TCNQ, DDQ and iodine (± 1 min) then recording the results. These changes did not affect significantly the measured response (absorbance) of LXP reaction products. RSD% of the measured absorbance for the studied variations did not exceed 1.6%. Table 3 presents the effect of the tested changes on the measured absorbance values.

Table 3: Study of robustness of the proposed spectrophotometric methods

Reagent	Parameter	Absorbance ± SD[a]	RSD %[b]
p-CA	**Reagent volume (mL)** 0.6 ± 0.10	0.641 ± 0.0046	0.72
	Working wavelength (nm) 523 ± 3	0.641 ± 0.0006	0.09
TCNQ	**Reagent volume (mL)** 1.6 ± 0.20	0.624 ± 0.0096	1.54
	Working wavelength (nm) 844 ± 3	0.603 ± 0.0146	2.42
	Reaction time (min) 10 ± 1	0.614 ± 0.0069	1.12
DDQ	**Reagent volume (mL)** 0.8 ± 0.10	0.575 ± 0.0057	0.99
	Working wavelength (nm) 458 ± 3	0.575 ± 0.0046	0.80
	Reaction time (min) 10 ± 1	0.585 ± 0.0042	0.72
PA	**Reagent volume (mL)** 0.6 ± 0.10	0.652 ± 0.0032	0.49
	Working wavelength (nm) 414 ± 3	0.642 ± 0.0021	0.33
Iodine	**Reagent volume (mL)** 0.8 ± 0.10	0.638 ± 0.0049	0.77
	Working wavelength (nm) 361 ± 3	0.631 ± 0.0066	1.05
	Reaction time (min) 10 ± 1	0.623 ± 0.0020	0.32

[a] Mean ± standard deviation for three determinations. [b] % Relative standard deviation.

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Stability of solutions

The stability of the colored products at room temperature was checked. Within 30 min after measurement No significant changes in absorbance readings were noticed. Additionally, when stored refrigerated at 4 ºC, the stock standard solutions of LXP in methanol were stable for at least 3 days

Assay of LXP dosage forms

The determination of LXP in its pharmaceutical formulations (tablets and capsules) was performed using the proposed colorimetric methods. The assay results declared satisfactory accuracy and precision as revealed from % recovery, SD and RSD% values (Table 4). The results of the assay of LXP dosage forms indicated that no interference was detected from commonly encountered excipients.

Estimation of LXP in its commercial products was also performed using a reference reported reversed phase HPLC method with UV detection [12]. For each pharmaceutical preparation, the one-way analysis of variance test (Single factor ANOVA) was used for the statistical comparison of the results of the proposed methods with those of the reference method [27]. The calculated F-values did not exceed the critical value, revealing that there were no significant differences between the proposed methods and the reference method (Table 4), and accordingly the proposed spectrophotometric methods are considered as accurate and precise as the reference HPLC method. From these results it is obvious that all the proposed methods are applicable to the analysis of LXP in its dosage forms with optimal and analogous analytical performance.

Table 4: Analysis of LXP in its pharmaceutical preparations using the proposed spectrophotometric methods and the reference method

Roxonin® 60 tablets						
Parameters	**p-CA**	**TCNQ**	**DDQ**	**PA**	**Iodine**	**Reference method**
%Recovery ± SD[a]	99.77±0.90	100.55±1.68	101.04±1.26	100.92±0.25	99.70±1.87	101.46±0.94
RSD %[b]	0.90	1.67	1.25	0.25	1.88	0.93
Single factor ANOVA	*F* = 1.58 , *F* (critical) = 2.62					
Roxogesic® tablets						
Parameters	**p-CA**	**TCNQ**	**DDQ**	**PA**	**Iodine**	**Reference method**
%Recovery ± SD[a]	101.28±1.22	99.60±1.37	101.26±0.98	99.86±1.16	100.32±1.46	101.34±0.77
RSD %[b]	1.20	1.38	0.97	1.16	1.46	0.76
Single factor ANOVA	*F* = 2.19 , *F* (critical) = 2.62					
Loujain® capsules						
Parameters	**p-CA**	**TCNQ**	**DDQ**	**PA**	**Iodine**	**Reference method**
%Recovery ± SD[a]	98.66±1.64	99.61±1.48	99.09±1.43	99.55±1.47	99.48±1.77	99.26±1.28
RSD %[b]	1.66	1.49	1.44	1.48	1.78	1.29
Single factor ANOVA	*F* = 0.28 , *F* (critical) = 2.62					

[a] Mean ± standard deviation for five determinations. [b] % Relative standard deviation.

CONCLUSION

For the first time, spectrophotometric analysis methods for the determination of loxoprofen sodium based on its electron donating ability were developed. These developed colorimetric methods have the advantages of being simple, sensitive and offering reasonable accuracy and sensitivity for the determination of loxoprofen sodium in the micro range. To the best of our knowledge, reviewing the literature revealed that no colorimetric methods were applied for the quantitative analysis of LXP and the vast majority of analytical

methods for its determination are based on HPLC. The developed spectrophotometric methods do not require elaborate treatment or the expensive and sophisticated experimental setup of other separation techniques.

The principal advantage is the simplicity of the proposed methods utilizing only a single step reaction and a single solvent and being free from interferences from the common excipients. Therefore, they can be used as general methods for the routine quality control assay of LXP in bulk powder and in pharmaceutical preparations. Nevertheless, the preference of some of these methods on the others is based on their sensitivity and experimental conditions (e.g. reaction time). For example, when rapid analysis is required faster methods involving p-chloranilic acid and picric acid are preferred since the color products are formed instantaneously and measured at zero time. In contrast, when high sensitivity is required on expense of the analysis time, the methods involving TCNQ and iodine are preferential.

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