Methods and objects of chemical analysis, 2024, Vol. 19, No. 2, 101–110

Simultaneous Spectrophotometric Determination of Piroxicam, Naproxen, Diclofenac Sodium and Mefenamic Acid in Pharmaceutical Formulations by Partial Least Squares Method

Ruaa M. Mahmood*, Samar A. Darweesh, Nahla A. Alassaf, Rokayia S. Al-Khalisy

Department of Chemistry, College of Education for Pure Science (Ibn Al-Haitham), University of Baghdad, Iraq; *e-mail: ruaa.m.m@ihcoedu.uobaghdad.edu.iq

Received: February 03, 2024; Accepted: March 07, 2024
DOI: 10.17721/moca.2024.101-110

A chemometric method, partial least squares regression (PLS) was applied for the simultaneous determination of piroxicam (PIR), naproxen (NAP), diclofenac sodium (DIC), and mefenamic acid (MEF) in synthetic mixtures and commercial formulations. The proposed method is based on the use of spectrophotometric data coupled with PLS multivariate calibration. The Spectra of drugs were recorded at concentrations in the linear range of 1.0 - 10 μg mL^{-1} for NAP and from 1.0 - 20 μg mL^{-1} for PIR, DIC, and MEF. 34 sets of mixtures were used for calibration and 10 sets of mixtures were used for validation in the wavelength range of 200 to 400 nm with the wavelength interval λ = 1 nm in methanol. This method has been used successfully to quantify drugs in pharmaceutical formulations with no interference from excipients. The proposed method is simple, quick, and can be used as an alternative analysis tool in drug and formulation quality control as well as process control.

Keywords: partial least squares method; piroxicam; naproxen; diclofenac sodium; mefenamic acid

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a class of drugs that are prescribed alone or in conjunction with other medications to treat a variety of clinical signs, including short and long-term pain states and a variety of musculoskeletal disorders [1]. Negative effects that are noticeable of NSAIDs include gastrointestinal bleeding, peptic ulcer disease, edema, renal insufficiency, and hypertension. Some NSAIDs have recently also been linked to a higher risk of myocardial infarction [2].

Piroxicam (PIR), is the first member of enolic acid class of NSAIDs which termed "oxicams" [3], chemically known as 4-Hydroxy-2-methyl-N-(pyridin-2-yl)-2H-1,2-benzothiazine-3-carboxamide 1,1-dioxide [4], Figure 1a. It is used for acute and long-term therapy to treat osteoarthritis and rheumatoid arthritis symptoms. It has also been used to treat acute gout because of its uricosuric action [5].

Naproxen (NAP) is a derivative of propionic acid NSAID with analgesic and antipyretic properties that is structurally related to ibuprofen. It is used to treat, menstrual cramps, pain, inflammatory diseases such as gout, rheumatoid arthritis and fever [6]. Chemically, (2S)-2-(6-Methoxynaphthalen-2-yl) propanoic acid [4], Figure 1b.

Diclofenac sodium (DIC) is an effective nonsteroidal pain reliever, chemically known as sodium 2-[(2,6-Dichlorophenyl)amino]phenyl]acetate [4], Figure 1c. It is primarily prescribed for the treatment of ankylosing spondylitis and osteoarthritis. Contrary to other NSAIDs, diclofenac has a relatively high therapeutic index [7].

Mefenamic acid (MEF) is a derivative of anthranilic acid [8], chemically known as 2-[(2,3-Dimethylphenyl) amino]benzoic acid [4], Figure 1d. Its uses are associated with low incidence of side effects without serious complications, and it has modest anti-inflammatory activity. It is also characterized by its rapid absorption, metabolism, and excretion [9].

Several methods have been reported, including spectrophotometry, HPLC, flow injection analysis, potentiometric, and so forth for simultaneous or individual quantification of PIR [10-15], NAP [16-25], DIC [26-33] and MEF [34-42]. To our knowledge, only one published article reported RP-HPLC technique for the determination of quaternary mixture of PIR, NAP, DIC, and MEF in pure and pharmaceutical formulation [43], but till now no published literature covers the simultaneous determination of these drugs by PLS method.

In 1972, world introduced chemometrics. Chemometrics is defined as the chemical discipline that employs statistical techniques and mathematical methods to design experiments or choose the optimal measurement procedures. They are also used to providing the most chemical information possible through analyzing chemical data. The importance of chemometric methods in the chemistry field has increased in recent years due to the availability of modern analytical techniques that can produce multivariate responses and overflow data for each

analyzed sample. Additionally, multivariate data cannot be resolved using conventional univariate statistics. As a result, analytical chemists focus on multivariate data analysis techniques based on chemometrics to uncover important information from noisy flood data. In chemometrics-assisted spectrophotometric measurement, the calibration set must be used for calibration, while the test set can be used for determination or prediction. Calibration sets are objects used to create models using the multivariate calibration method, whereas test sets are objects of unknown samples that are subjected to prediction of their contents. Multivariate calibration methods come in at least three types: partial least square regression, principal component regression, and multiple linear regression [44-46]. This work presents PLS method for simultaneous spectrophotometric determination of PIR, NAP, DIC and MEF in pure form and pharmaceutical preparations. The benefit of using multi-component analysis with multivariate calibration is how quickly the components in a mixture can be determined, avoiding the need for a preliminary separation step.

Materials and Methods

Apparatus. A Schimadzu double beam UV-Visible spectrophotometer 1800 (Kyoto-Japan) equipped with 10 mm quartz cell. The UV Probe Software version: 2.42 was employed to acquire of the data.

Software. A set of calibration mixture was prepared using a Multilevel Categoric Design (create by Design-Expert version:13) for the determination of PIR, NAP, DIC and MEF simultaneously, while (PLS) analysis was performed using the OriginPro 2021 software (version: 9.8).

Chemicals. The standard grade powders of Piroxicam, Naproxen, Diclofenac sodium and Mefenamic acid were provided as a gift in pure form (99.99 %) by the State Company for Drug Industries and Medical Appliances Samara-Iraq (SDI) for use in this work. Methanol 99.9 % (Scharlau, Turkey).

Commercial Pharmaceutical formulations of different brands analyzed in this investigation were obtained from local pharmacies; Piroxicam 20 mg / capsule (India), Naproxen 500 mg / tablet (India), Epifenac 75 mg/3 mL ampoule (Egypt) and Mefenamic acid 250 mg / capsule (China).

NSAIDs standard solution. The standard solutions of PIR, NAP, DIC and MEF were prepared separately by dissolving 0.1 g in 100 mL methanol. The stock solutions were shielded from light and stored at 4ºC.

Procedures

Calibration. Multilevel categoric design (three levels for NAP and five levels for PIR, DIC and MEF) was employed in the construction of the training set. Thirty-four samples were prepared for calibration set. An aliquot of the four working solutions containing 5-50 µg for NAP and 5-100 µg for PIR, DIC, and MEF were placed in and the volume made up to 5 mL with methanol.

The UV absorption spectra in wavelength range 200-400 nm were recorded against solvent blank, with a 10 nm sec^{-1} scan speed, bandwidth of 1 nm and the spectra data points were collected at 1 nm intervals.

Validation. A validation set of 10 samples were created to evaluate the effectiveness of PLS method.

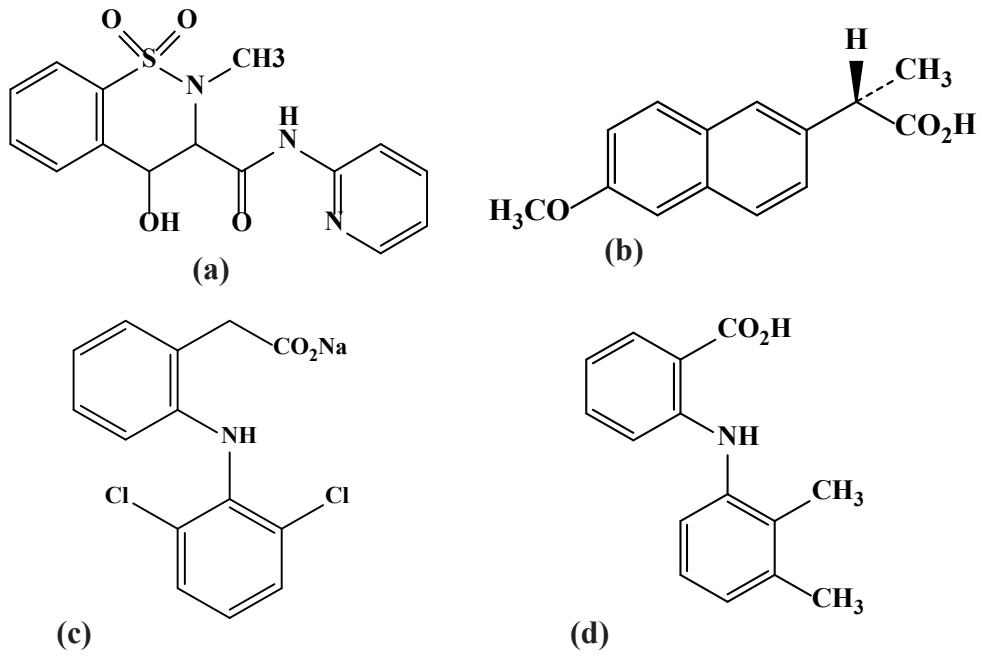

Figure 1. The structure formulas of four compounds: (a) Piroxicam, (b) Naproxen, (c) Diclofenac sodium and (d) Mefenamic acid.

Analysis of PIR, NAP, DIC, and MEF in commercial preparations. Ten tablets for NAP and content of ten capsules for PIR and MEF were precisely weighed individually, and the weight average was determined. The tablets were grinded into a powdered form then mixed well. An amount of powder equal to 0.1454 g, 0.012 g and 0.0451 g for PIR, MEF and NAP, respectively, was accurately weighed and dissolved in a small amount of methanol and stirred for 10 min to ensure that the drug was completely dissolved before being transferred into a 10 mL volumetric flask for PIR and MEF and 25 mL volumetric flask for NAP and diluted to the mark with methanol to get 1000 μg mL^{-1}. Before use, the solutions were filtered through Whatman filter paper No.41 to remove any suspended or undissolved material. While DIC was prepared from the injection ampoule (each 3 mL contains 75 mg of DIC). To make 1000 μg mL^{-1} DIC solution, an accurately measured volume of 0.4 mL was transferred into a 10 mL volumetric flask and diluted to the mark with methanol. Freshly prepared working solutions were analyzed using the suggested procedure.

Results and Discussion

Absorption spectra

The UV absorption spectra of PIR, NAP, DIC and MEF were recorded over the wavelength range of 200-400 nm. As shown in Figure 2 there is a distinct overlap between the drugs, this spectral overlapping prevents resolving of the mixtures by univariate analysis methods. So, simultaneous multicomponent analysis via PLS method was used for this purpose.

Individual calibrations

In order to select a concentration range suitable for spectrophotometric measurements (for each individual compound), the linearity of the maximal signals was investigated. The absorption values were measured at λ_{max} of 327, 231, 282 and 280 nm with different concentrations of the PIR, NAP, DIC and MEF, respectively. The linearity of each compound was determined by plotting absorbance values against drug concentrations and were found to be 1.0 - 10 μg mL^{-1} for NAP and 1.0 - 20 μg mL^{-1} for PIR, DIC and MEF Figure 3.

Selection the optimal number of factors for PLS method

A cross validation technique that eliminates one sample at a time [47] was applied to choose the optimum number of factors in PLS algorithm. This was accomplished by randomly excluding one sample at a time and allowing the software to estimate the residual error sum of squares value (PRESS = $\sum(-)^2$) (where, is known concentration and is the predicted concentration of analyte) and use it to assess the suitability of model [48]. Figure 4, shows directly how the root mean PRESS is reached and stabilize at 7 factors and then slightly change, indicating that 7 is the optimal number of factors.

Calibration and validation sets

A training set of 34 mixtures was created from spectrophotometric data by performing careful UV measurements on a set of known samples. This calibration set was used to build up PLS regression mode after organizing the data into a matrix that was divided into pairs. In other words, each absorbance matrix was paired with its respective concentration matrix using OriginPro 2021 software, Table 1 show the concentrations of the training set and their predictions. This set used to examine the predictive abilities of PLS model for simultaneous determination of PIR, NAP, DIC and MEF in every mixture. A validation set of ten quaternary mixtures were randomly selected and analyzed with the developed PLS method Table 2. The prediction error of a single component in the mixture was calculated using the relative standard error (R.S.E.) of the prediction concentration [49].

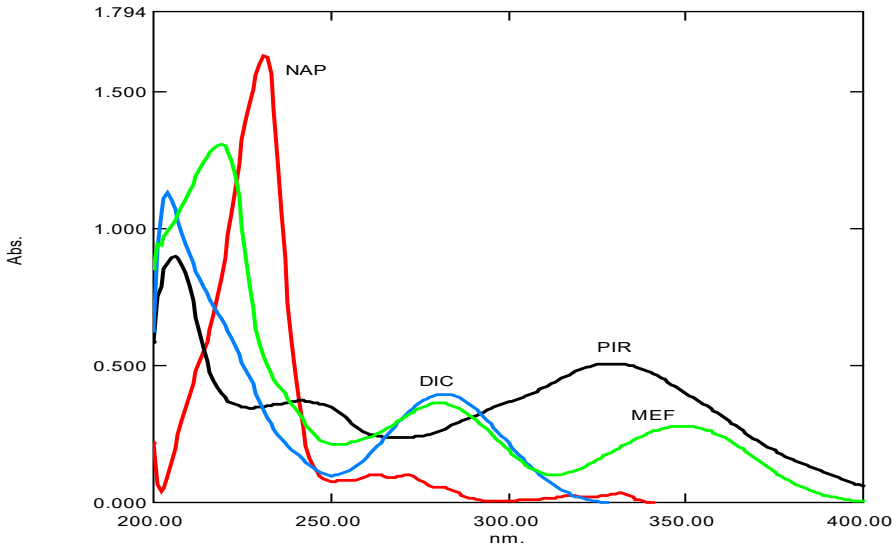

Figure 2. The spectra of 10 μg.mL^{-1} of PIR, DIC, and MEF and 5 μg.mL^{-1} of NAP against their solvent blanks.

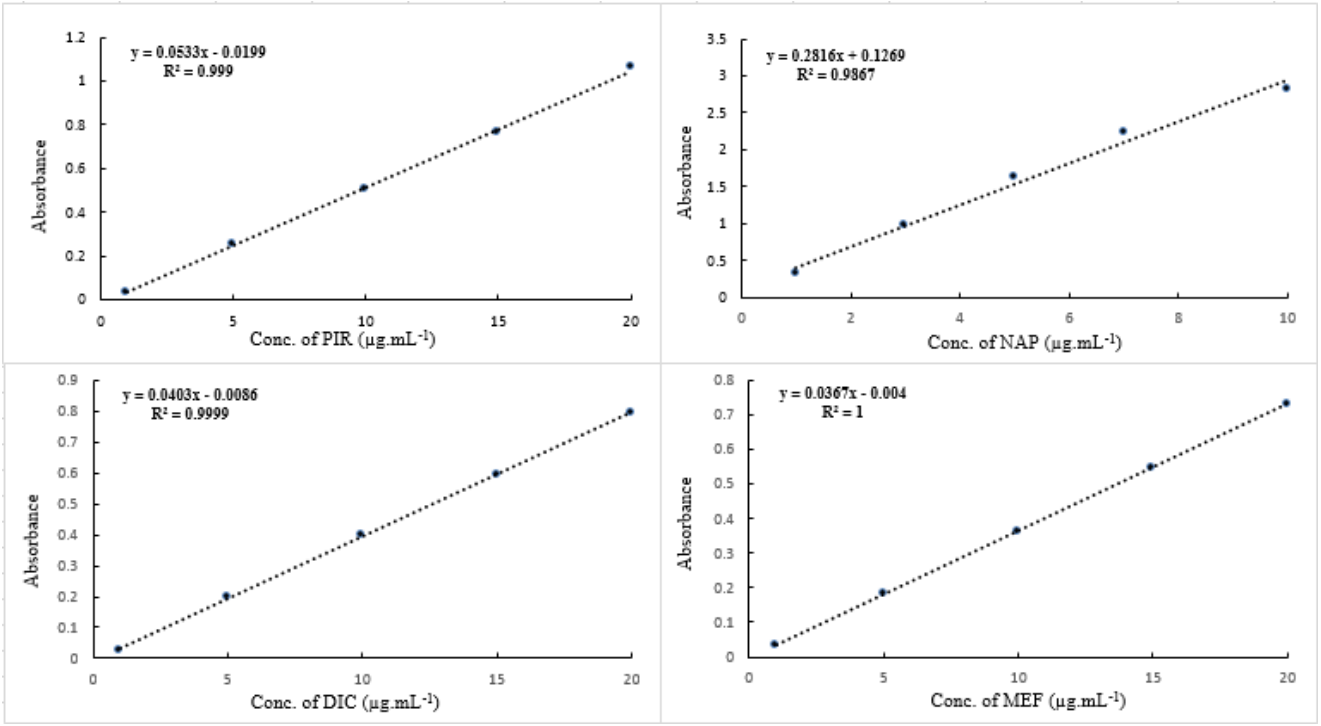

Figure 3. Linear dynamic concentration (LDC) ranges for PIR, NAP, DIC and MEF.

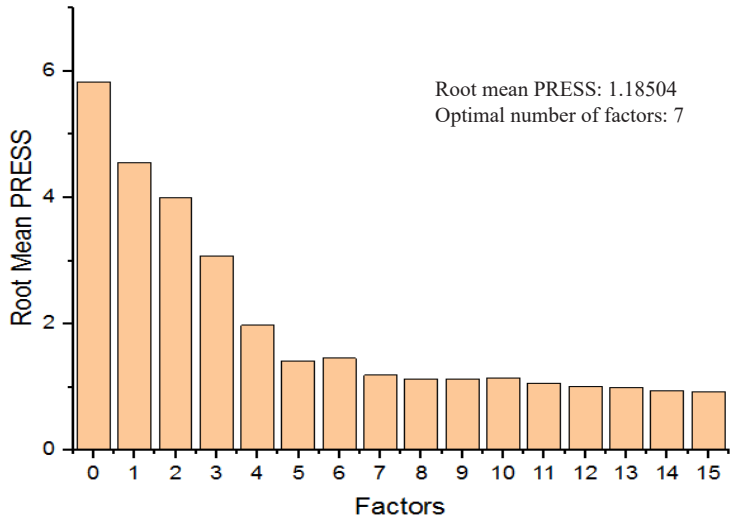

Figure 4. Plot of Root Mean PRESS vs number of factors for 34 PIR, NAP, DIC and MEF mixtures.

$$RSE\ (\%) = \sqrt[2]{\frac{\sum_{i=1}^{N}(\hat{y}_i - y_i)^2}{\sum_{i=1}^{N}(y_i)^2}} \times 100$$

where, N is the number of samples, y_i concentration of the component in the ith mixture and $\hat{y}_i$ is the estimated concentration.

The root mean squares error of calibration (RMSEC) and root mean squares error of predictions (RMSEP) which were diagnostic test of the average error in the predicted concentrations for each component [50].

$$RMSEC = \sqrt[2]{\frac{\sum_{i=1}^{N}(\hat{y}_i - y_i)^2}{n}}$$

$$RMSEP = \sqrt[2]{\frac{\sum_{i=1}^{N}(\hat{y}_i - y_i)^2}{m}}$$

where, n and m are the number of samples in calibration set and test set, respectively.

Moreover, slop and correlation coefficient (r) between measured and predicted concentrations in calibration set and validation set are given in Table 3.

Table 1. Concentrations of training set and their predictions by PLS model.

Mixture (μg mL^{-1})				Prediction (μg mL^{-1})				Recovery %			
PIR	**NAP**	**DIC**	**MEF**	**PIR**	**NAP**	**DIC**	**MEF**	**PIR**	**NAP**	**DIC**	**MEF**
1	1	10	1	1.02018	1.04976	10.2594	1.30968	102.018	104.976	102.594	130.968
10	1	1	1	10.42963	1.04363	1.16712	1.49437	104.2963	104.363	116.712	149.437
10	10	5	10	9.99659	10.01696	4.95153	10.36305	99.9659	100.1696	99.0306	103.6305
10	5	10	10	10.06819	4.97197	10.3634	9.74433	100.6819	99.4394	103.634	97.4433
1	10	1	1	0.86389	10.02426	1.42248	1.78474	86.389	100.2426	142.248	178.474
10	10	10	5	10.20707	10.02398	10.00027	4.46317	102.0707	100.2398	100.0027	89.2634
5	5	5	20	4.95101	5.0062	5.00068	20.21162	99.0202	100.124	100.0136	101.0581
10	10	10	10	9.95063	10.00701	9.86321	9.93161	99.5063	100.0701	98.6321	99.3161
5	1	1	1	4.53446	0.92128	0.88735	1.13165	90.6892	92.128	88.735	113.165
1	1	1	10	1.31412	0.88906	1.19783	9.22431	131.412	88.906	119.783	92.2431
15	1	1	1	15.03577	0.97071	1.04073	1.6777	100.2385	97.071	104.073	167.77
10	10	20	10	10.01515	9.96807	19.97181	10.24646	100.1515	99.6807	99.85905	102.4646
1	1	1	1	0.6596	1.08202	0.85806	0.82538	65.96	108.202	85.806	82.538
5	5	20	5	4.90093	5.08023	19.99076	5.01862	98.0186	101.6046	99.9538	100.3724
1	5	5	5	0.83407	5.02123	4.64053	4.43895	83.407	100.4246	92.8106	88.779
20	10	10	10	20.02678	10.02545	10.13575	9.74366	100.1339	100.2545	101.3575	97.4366
5	5	10	5	5.21142	5.0345	10.3223	4.66849	104.2284	100.69	103.223	93.3698
1	1	20	1	1.08388	0.92234	19.91708	1.06023	108.388	92.234	99.5854	106.023
10	5	5	5	10.36635	4.939	4.6844	4.94457	103.6635	98.78	93.688	98.8914
1	1	5	1	1.37904	1.08717	4.87176	0.78057	137.904	108.717	97.4352	78.057
1	10	10	10	1.08245	9.92544	10.45361	9.78325	108.245	99.2544	104.5361	97.8325
15	5	5	5	14.64471	5.00561	4.39961	5.2029	97.6314	100.1122	87.9922	104.058
15	5	15	15	14.86425	5.00562	14.83115	15.35057	99.095	100.1124	98.87433	102.3371
20	1	1	1	19.9583	1.00435	1.33016	0.53121	99.7915	100.435	133.016	53.121
5	1	5	5	4.87503	0.92187	4.75578	4.57075	97.5006	92.187	95.1156	91.415
1	1	15	1	0.92721	1.02431	14.96112	1.39822	92.721	102.431	99.7408	139.822
10	1	10	10	9.69293	0.98968	9.95096	10.013	96.9293	98.968	99.5096	100.13
5	10	10	10	4.81138	9.95383	9.46781	10.26184	96.2276	99.5383	94.6781	102.6184
5	5	5	10	5.23164	4.94961	5.19091	9.80417	104.6328	98.9922	103.8182	98.0417
10	10	15	10	9.98117	9.98159	14.82988	9.8664	99.8117	99.8159	98.86587	98.664
15	1	15	15	14.946	1.03275	15.13309	15.22704	99.64	103.275	100.8873	101.5136
15	10	15	15	15.03331	10.05715	15.15687	14.81539	100.2221	100.5715	101.0458	98.76927
20	1	20	20	20.0574	0.973	20.00797	19.85301	100.287	97.3	100.0399	99.26505
1	1	1	20	1.04549	1.09039	0.98461	20.25908	104.549	109.039	98.461	101.2954
Mean recovery								100.4537	100.0103	101.934	104.6936
RSE %								2.0433	0.8547	2.3038	3.6798

Table 2. Concentrations of validation set and their predictions by PLS model.

Mixture (μg mL^{-1})				Prediction (μg mL^{-1})				Recovery %			
PIR	**NAP**	**DIC**	**MEF**	**PIR**	**NAP**	**DIC**	**MEF**	**PIR**	**NAP**	**DIC**	**MEF**
10	10	10	20	10.87487	10.45173	10.66573	18.94825	108.7487	104.5173	106.6573	94.74125
10	10	1	10	9.86889	9.73942	0.96439	10.52665	98.6889	97.3942	96.439	105.2665
5	5	5	5	4.52999	4.91894	4.72942	4.6173	90.5998	98.3788	94.5884	92.346
15	10	10	10	14.75495	10.16035	9.43707	10.79007	98.3663	101.6035	94.3707	107.9007
20	5	5	5	19.86321	4.814	4.12454	4.50383	99.31605	96.28	82.4908	90.0766
5	5	5	1	5.05323	4.92734	4.79335	0.77114	101.0646	98.5468	95.867	77.114
5	5	15	5	4.95517	4.97254	15.42486	5.44677	99.1034	99.4508	102.8324	108.9354
20	5	20	20	20.69367	5.71189	20.70975	19.23137	103.4684	114.2378	103.5488	96.15685
5	10	5	5	4.31242	9.81575	4.79018	5.34539	86.2484	98.1575	95.8036	106.9078
20	10	20	20	20.74923	10.03824	20.72885	19.83457	103.7462	100.3824	103.6443	99.17285
Mean recovery								98.93507	100.8949	97.62422	97.8618
RSE %								3.884112	3.76738	4.679148	4.75224

Table 3. Summary of the statistical parameter values of the developed PLS model.

	Variable			
Parameter	**PIR**	**NAP**	**DIC**	**MEF**
Spectral range (nm)	200-400	200-400	200-400	200-400
Conc. range (μg mL^{-1})	1-20	1-10	1-20	1-20
calibration set				
RMSEC	0.204633	0.051615	0.244608	0.354757
Slope	1	1	1	1
Correlation coefficient (r)	0.9995	0.9999	0.9992	0.9982
validation set				
RMSEP	0.510136	0.297838	0.538814	0.582222
Slope	0.9563	0.9919	0.9394	1.0311
Correlation coefficient (r)	0.9978	0.9932	0.9985	0.9970

As in Table 3, small values of RMSEC, the relating measure for model fit, were acquired. The slopes of the plots between true and predicted concentrations for the four drugs in the calibration set were found to be 1, which indicate that predicted concentrations did not deviate from true concentrations. Furthermore, the correlation coefficient (r) was greater than 0.999 for PIR, NAP and DIC and greater than 0.998 for MEF, indicating a high correlation between real and predicted concentrations, Figure 5. the developed model ability to predict the test set, which was not included in the model construction, showed low values for the root mean square error of prediction (RMSEP). As in Figure 6, the slops of the four plots of true versus predicted concentrations in the validation set were closed to 1 and the correlation coefficients (r) were higher than 0.993. These results amply demonstrated how accurately the resulting model was able to predict new samples.

Accuracy and Precision

The accuracy and precision of the developed PLS model for the determination of PIR, NAP, DIC, and MEF simultaneously in quaternary mixtures were tested by using two different concentrations of each drug. The results in Table 4 show that the method has good accuracy and precision values for each concentration level.

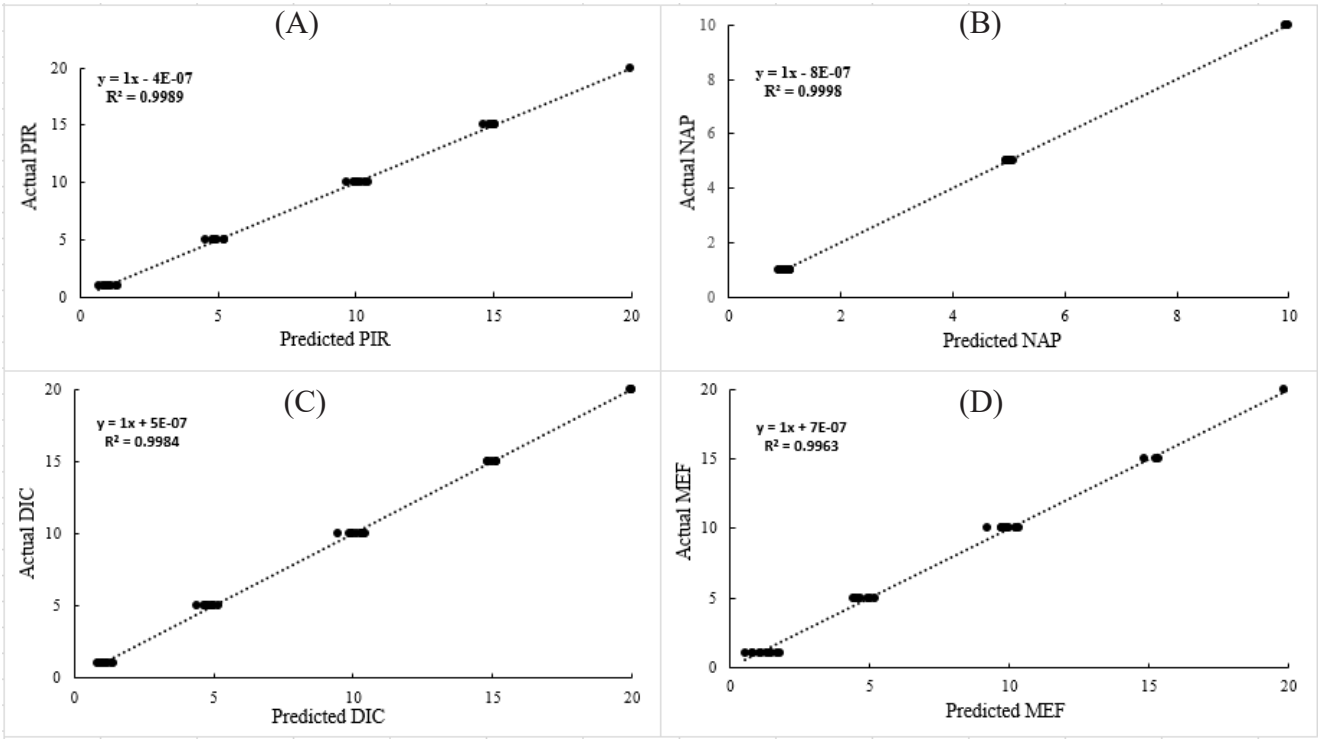

Figure 5. Plots of predicted and actual concentration of calibration set: (A) Piroxicam, (B) Naproxen, (C) Diclofenac sodium and (D) Mefenamic acid.

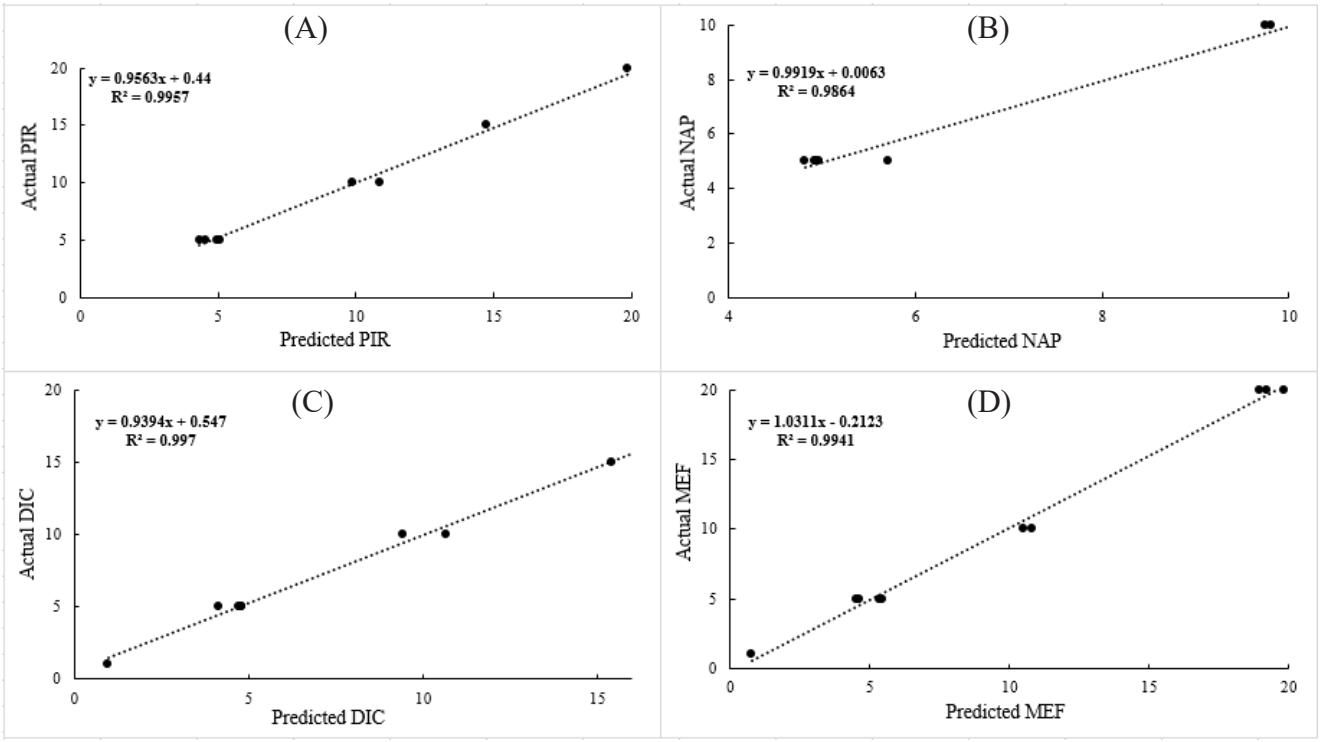

Figure 6. Plots of predicted and actual concentration of validation set: (A) Piroxicam, (B) Naproxen, (C) Diclofenac sodium and (D) Mefenamic acid.

Application to pharmaceutical formulations

The proposed method was successfully applied in several pharmaceutical preparations (capsule, tablet, and ampoule) for the determination of the drugs. Table 5 shows the results of three replicate measurements. The results show a good agreement between the estimated values and the manufacturer's label claims in the formulation.

Table 4. Accuracy and precision of the suggested PLS method.

		(μg mL^{-1})					
Drug	**Taken**		**Found**		**Mean**	**RE %**	**RSD %**
PIR	5	5.1315	5.0209	4.7345	4.9623	-0.7544	4.1292
	10	10.2406	10.1811	10.2100	10.2106	2.1058	0.2914
NAP	5	5.1528	5.1449	5.1344	5.1440	2.8807	0.1793
	10	10.1025	10.0469	10.1908	10.1134	1.1340	0.7176
DIC	5	5.1998	5.2642	5.1732	5.2124	4.2471	0.8976
	10	10.2153	10.6445	10.6202	10.4933	4.9332	2.2978
MEF	5	4.8810	4.7532	4.7247	4.7863	-4.2743	1.7388
	10	10.0038	10.3720	9.9205	10.0988	0.9877	2.3790

Table 5. Results of analysis of pharmaceutical preparations by the proposed method.

		Weight (mg)					
Sample	**Labeled**		**Found**		**Mean**	**Recovery %**	**C.V. %**
Piroxicam (capsule)	20	21.582	21.819	21.628	21.676	108.382	0.579
Naproxen (tablet)	500	507.740	510.260	506.175	508.058	101.612	0.406
Epifenac (ampoule)	75	66.908	68.214	66.9081	67.343	89.791	1.120
Mefenamic acid (capsule)	250	257.800	255.032	259.8438	257.559	103.024	0.938

Conclusion

The present method was developed by combining electronic absorption measurements with PLS multivariate analysis. This method offers high sample throughput, suitable for routine quality control and doesn't require complex sample treatments or time-consuming separations. Moreover, the proposed method yielded high precision results, allowing for simple, economical, and accurate simultaneous determination of four nonsteroidal anti-inflammatory drugs (PIR, NAP, DIC and MEF) in their pure powder and pharmaceutical formulations.

References

1. McGettigan, P. & Henry, D. Use of non-steroidal anti-inflammatory drugs that elevate cardiovascular risk: an examination of sales and essential medicines lists in low-, middle-, and high-income countries. *PLoS Med.* **10**, e1001388 (2013).

2. K. Peterson, M. McDonagh, D. S. Thakurta, T. Dana, C. Roberts, R. C. and M. H. Drug class review: non-steroidal anti-inflammatory drugs (NSAIDs). *Oregon Heal. Sci. Univ.* (2010).

3. Henry, P. M. and D. J. K. Aronson. *Lippincott Williams & Wilkins* 7th ed., 1018 (2013).

4. Pharmacopoeia, B. CD. Room, Majes's Stationary Office, London, BP, Co.-UK. (2012).

5. Alagarsamy, V. Textbook of medicinal chemistry. *Elsevier Heal. Sci.* **2**, 92 (2010).

6. Ogle, H. D. and O. E. Oral surgery for the general dentist", Elsevier Health Sciences. 108 (2012).

7. Nayak, A. K. Thermodynamic study of the diclofenac sodium solubility in various oils. *Chemistry (Easton).* **19**, 121–128 (2010).

8. Aronson, J. K. *Meyler's side effects of analgesics and anti-inflammatory drugs*. (elsevier, 2009).

9. Phaliwong, P. & Taneepanichskul, S. The effect of Mefenamic acid on controlling irregular uterine bleeding second to Implanon use. *J. Med. Assoc. Thailand= Chotmaihet Thangphaet* **87**, S64-8 (2004).

10. Hadi, R. I. A. and H. The influence of some amino acids, vitamins and anti-inflammatory drugs on activity of chondroitinase produced by Proteus vulgaris caused urinary tract infection. *Iraqi J. Sci.* 2412–2421 (2016).

11. Abed, R. I. & Hadi, H. Direct determination of piroxicam in pharmaceutical forms using flow injection-spectrophotometry. *Bull. Chem. Soc. Ethiop.* **34**, 13–23 (2020).

12. Calvo, A. M. *et al.* Effective method for the detection of piroxicam in human plasma using HPLC. *Braz. Oral Res.* **30**, (2016).

13. Celebier, M. *et al.* Determination of the Physicochemical Properties of Piroxicam. *Turkish J. Pharm. Sci.* **17**, 535 (2020).

14. Caet, M. P. *et al.* Pharmacopoeial HPLC methodology improvement: A case study of piroxicam. *Drug Anal. Res.* **4**, 50–57 (2020).

15. Rajendraprasad, N. & Basavaiah, K. Potentiometric determination of piroxicam and oxfendazole in pharmaceuticals. *Curr. Chem. Lett.* **5**, 33–46 (2016).

16. Yousif, H. S. & Khalil, Y. I. In situ gelling formulation of Naproxen for oral sustained delivery system. *Iraqi J. ofPharmceutical Sci.* **18**, 13–20 (2009).

17. Mahmood, W. A. R. Preparation and Characterization some New of Naproxen Drug Derivatives. *Baghdad Sci. J.* **14**, 564–574 (2017).

18. Hasan, G. A. & Bakir, M. H. Spectrophotometric Determination of Naproxen using Metol reagent by Oxidative Coupling Reaction. in *IOP Conference Series: Materials Science and Engineering* vol. 1058 12079 (IOP Publishing, 2021).

19. Barazandeh, A., Najafpour, G. D., Alihosseini, A., Kazemi, S. & Akhondi, E. Spectrophotometric Determination of Naproxen Using Chitosan Capped Silver Nanoparticles in Pharmaceutical Formulation. *Int. J. Eng.* **34**, 1576–1585 (2021).

20. Al-Sarraj, T. Z. & Al-Taee, A. M. A Green Indirect Spectrophotometric Estimation Of The Analgesic Naproxen In The Existence Of The Another Analgesic Paracetamol. *Egypt. J. Chem.* **65**, 1–2 (2022).

21. Zayed, M. A. & EL-Gizouli, A. M. M. Spectroscopic Studies of Naproxen and Indomethacin Reactions with Copper (II) Reagent and their Micro Determination through Ion-Pair Formation and their Biological Activities. (2019).

22. Tsvetkova, B. RP-HPLC method for determination of naproxen in pharmaceutical dosage form. *Pharmacia* **62**, 15–19 (2015).

23. Patel, P. N., Samanthula, G., Shrigod, V., Modh, S. C. & Chaudhari, J. R. RP-HPLC method for determination of several NSAIDs and their combination drugs. *Chromatogr. Res. Int.* **2013**, (2013).

24. Wang, L.-J., Tang, Y.-H. & Liu, Y.-H. Flow injection chemiluminescence determination of loxoprofen and naproxen with the acidic permanganate-sulfite system. *J. Pharm. Anal.* **1**, 51–56 (2011).

25. Lenik, J. & Łyszczek, R. Functionalized β-cyclodextrin based potentiometric sensor for naproxen determination. *Mater. Sci. Eng. C* **61**, 149–157 (2016).

26. Dikran, S. B. & Mahmood, R. M. Spectrophotometric Determination of Diclofenac sodium Using 2, 4-dinitrophenylhydrazine in Pure Form and Pharmaceutical Preparations. *Ibn AL-Haitham J. Pure Appl. Sci.* **28**, 129–141 (2017).

27. Mukkawi, R., Shantier, S. & Gadkariem, E. A. Spectrophotometric Method for the Simultaneous Analysis of Diclofenac Sodium and Lidocaine Hydrochloride in Bulk and Dosage Forms. *Pharm. Chem. J.* **55**, 831–834 (2021).

28. Darweesh, S. A., Khalaf, H. S., Al-Khalisy, R. S., Yaseen, H. M. & Mahmood, R. M. Advancement and validation of new Derivative spectrophotometric method for individual and simultaneous estimation of Diclofenac sodium and nicotinamide. *Orient. J. Chem.* **34**, 1625 (2018).

29. Jalbani, N. S. *et al.* Gas Chromatographic and Spectrophotometric Determination of Diclofenac Sodium, Ibuprofen, and Mefenamic Acid in Urine and Blood Samples. *Turkish J. Pharm. Sci.* **17**, 465 (2020).

30. Alquadeib, B. T. Development and validation of a new HPLC analytical method for the determination of diclofenac in tablets. *Saudi Pharm. J.* **27**, 66–70 (2019).

31. Majeed, B. A. A., Muhseen, R. J. & Jassim, N. J. Adsorption of diclofenac sodium and ibuprofen by bentonite polyureaformaldehyde thermodynamics and kinetics study. *Iraqi J. Chem. Pet. Eng.* **19**, 29–43 (2018).

32. Kadhim, M. M. & Kubba, R. M. Theoretical investigation on reaction pathway, biological activity, toxicity and NLO properties of diclofenac drug and its ionic carriers. *Iraqi J. Sci.* 936–951 (2020).

33. Mahanty, B. & John, A. P. Development of Robust Partial Least Squares Regression Model for Spectroscopic Determination of Diclofenac Sodium in Environmental Samples. *Curr. Anal. Chem.* **16**, 241–249 (2020).

34. Wasito, H., Purnamasari, D. & Fareza, M. S. Mefenamic acid determination in tablet formulations using a selective and accurate spectrophotometric method based on prussian blue formation. *Malaysian J. Anal. Sci.* **25**, 71–80 (2021).

35. Saleem, B. A. & Alnuaimy, L. A. Spectrophotometric Determination of Mefenamic acid in pure and Pharmaceutical Preparations. in *Journal of Physics: Conference Series* vol. 1853 12048 (IOP Publishing, 2021).

36. Al-Awadie, N. S. T. & Al-Saeedi, M. K. K. Determination of Mefenamic Acid Using a New Mode of Irradiation (Array of Six Identical LEDs) and Detection (Twin Solar Cells) Through Turbidity Measurement by CFIA. *Iraqi J. Sci.* 1052–1070 (2016).

37. Al Abachi, M. Q. & Hadi, H. Simple, rapid and sensitive method for the determination of mefenamic

acid in pharmaceutical preparations. *J. Anal. Chem.* **69**, 769–776 (2014).

38. Al-Ameer, L., Hashim, K. K. & Taha, D. N. Determination of mefenamic acid in aqueous solutions using merging zone–continuous flow injection. *Water Pract. Technol.* **17**, 1881–1892 (2022).

39. Hamed, M. & Hammood, M. K. Simultaneous Determination of Trace Mefenamic Acid in Pharmaceutical Samples via Flow Injection Fluorometry. *Int. J. Drug Deliv. Technol* **10**, 395–401 (2020).

40. Alsafi, M. H. A. & Farhan, M. S. Synthesis, Characterization and Acute Anti-inflammatory Evaluation of New Mefenamic Acid Derivatives Having 4-Thiazolidinone Nucleus. *Iraqi J. Pharm. Sci. (PISSN 1683-3597, E-ISSN 2521-3512)* **28**, 138–146 (2019).

41. Kormosh, Z. A., Matviichuk, O. Y., Antal, I. P. & Bazel, Y. R. Sensors Based on Single-and Double-Layer Plasticized Membranes for the Potentiometric Determination of Mefenamic and Phenylanthranylic Acids. *J. Anal. Chem.* **75**, 820–828 (2020).

42. Al-Ghani, A. M., Alabsi, A. T. & Albaser, N. Simultaneous Spectrophotometric Estimation of Etamsylate and Mefenamic Acid in Presence of Etamsylate Main Impurities (Hydroquinone). *J. Chem. Pharm. Res.* **13**, 22–29 (2021).

43. Dikran, S. B. & Mahmood, R. M. High performance liquid chromatographic method for the determination of piroxicam, naproxen, diclofenac sodium, and mefenamic acid in bulk drug and pharmaceutical preparations. *J. Univ. Babylon Pure Appl. Sci.* **26**, 387–399 (2018).

44. Thomas, E. V. A primer on multivariate calibration. *Anal. Chem.* **66**, 795A-804A (1994).

45. Geladi, P. Chemometrics in spectroscopy. Part 1. Classical chemometrics. *Spectrochim. Acta Part B At. Spectrosc.* **58**, 767–782 (2003).

46. Lavine, B. & Workman, J. Chemometrics. *Anal. Chem.* **80**, 4519–4531 (2008).

47. Espinosa-Mansilla, A., Salinas, F. & Paya, I. D. O. Simultaneous determination of sulfadiazine, doxycycline, furaltadone and trimethoprim by partial least squares multivariate calibration. *Anal. Chim. Acta* **313**, 103–112 (1995).

48. Afkhami, A. & Sarlak, N. Simultaneous determination of salicylamide and paracetamol by spectrophotometric H-point standard addition method and partial least squares regression. *Acta Chim. Slov* **52**, 98–103 (2005).

49. Niazi, A. & Yazdanipour, A. Spectrophotometric simultaneous determination of nitrophenol isomers by orthogonal signal correction and partial least squares. *J. Hazard. Mater.* **146**, 421–427 (2007).

50. Ghasemi, J. B. & Zolfonoun, E. Simultaneous spectrophotometric determination of trace amounts of uranium, thorium, and zirconium using the partial least squares method after their preconcentration by α-benzoin oxime modified Amberlite XAD-2000 resin. *Talanta* **80**, 1191–1197 (2010).