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Research Article

MULTIVARIATE CHEMOMETRIC MODELS USED FOR THE ANALYSIS OF RAFOXANIDE IN PRESENCE OF ITS ALKALINE DEGRADATION PRODUCT

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ABSTRACT

Objective: Development and validation of three simple, accurate and precise chemometric models for the determination of rafoxanide in the presence of its alkaline degradation product without preliminary separation**. Methods:** These methods are classical least square (CLS), principal component regression(PCR) and partial least square (PLS-1). A 2-factor 5-level experimental design was built leading to 25 mixtures containing different ratios of rafoxanide and its alkaline degradation product. Thirteen mixtures were used as a training set, and the other twelve were used as a validation set. **Results**: Using of multi-wavelengths instead of the single wavelength spectrophotometry has greatly improved the precision and predictive abilities of these multivariate calibrations**. Conclusion:** The proposed methods have been found to be accurate, precise and can be used for determination of the drug in pure form and pharmaceutical formulations as well as in the presence of its degradation product without any preliminary separation steps.

Keywords: Rafoxanide; Chemometric methods; CLS; PCR; PLS-1.

INTRODUCTION

Rafoxanide is 3′-chloro-4′-(4-chlorophenoxy)-3,5-di-iodosalicyl-anilide (**Figure 1**). It is an anthelmintic used in veterinary medicine for the treatment of fascioliasis in cattle and sheep [1].Literature survey reveals that rafoxanide was determined by several techniques including colorimetry [2], UV-Spectrophotometry [3], GC [4], TLC [5,6], HPLC [6-9]and UPLC [5].

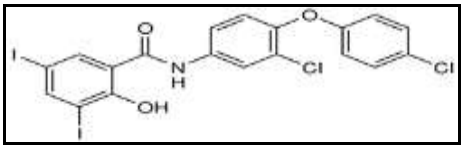

Fig. 1: Structural formula of rafoxanide.

This work aims to develop and validate simple, sensitive, selective and cost effective chemometric methods for the determination of rafoxanide in the presence of its alkali-induced degradation product without preliminary separation these methods namely classical least-squares (CLS), principal component regression (PCR), and partial least-squares (PLS-1)

EXPERIMENTAL

Instruments

Shimadzu dual beam UV-Visible 1800 Spectrophotometer,(Tokyo, Japan), equipped with 10 mm matched quartz cells.

Software

UV-Probe personal spectroscopy software version2.1. (SHIMADZU). All chemometric methods were implemented in Matlab R2013b (8.2.0.701), using PLS toolbox software version 2.1. The *t*-test and *F*-test were performed using Microsoft_ Excel.

Samples

Both pure rafoxanide (99.8%) (B. NO.RF/110315) and Flukanil® injection(B. NO. 1509104) were kindly supplied by Pharma-Swede, Egypt. 10th of Ramadan city, Egypt.

Chemicals and solvents

Hydrochloric acid, sodium hydroxide and methanol (El-Nasr Co., Egypt).The solvent used was methanol.

Standard solution

A stock solution of rafoxanide (100 µg/mL) was prepared by dissolving 10 mg of rafoxanide in 50 mL of methanol and complete to 100 mL with the same solvent.

Degraded sample

Accelerated alkali-induced degradation was performed by refluxing 100 mg of pure rafoxanide with 50 mL of 1 N sodium hydroxide solution for 7 hours. The solution was cooled to room temperature then neutralized to pH seven by addition of 1 N hydrochloric acid solution, and then evaporated to dryness under vacuum. The obtained residue was extracted with methanol (3 x 25 mL), filtered into a 100-mL volumetric flask and diluted to volume with methanol to obtain a stock solution labeled to contain degradate derived from 1 mg/mL of rafoxanide. Working solution of degradate (100 µg/mL) was obtained by further dilution of the stock solution with the methanol.

PROCEDURES

Experimental design for chemometric methods

A 5-level, 2-factor design was performed using five concentration levels for the drug and its alkaline degradate to be analyzed. The design spans the mixture space fairly well; where there are 5 mixtures for each compound at each concentration level, resulting in 25 mixtures. The central level of the design is 10µg/ml for both drug and degradate. **Table 1** represents the concentration design matrix. The absorption spectra of the prepared 25 mixtures were recorded over the wavelength range 225-415 nm with 1 nm interval. Thus the produced spectral datamatrix has 25 rows representing different samples and 191 columns representing wavelengths (25 x 191). Thirteen mixtures of this design were used as a calibration set and the other 12 mixtures were used as a validation set to test the predictive ability of the developed multivariate models.

Table 1: Experimental design of concentrations of rafoxanide and degradate mixtures used in chemometric methods.

Mix. No	Rafoxanide(µg/mL)	Degradate(µg/mL)
1	10	10
2	10	8
3	8	8
4	8	12
5	12	9
6	9	12
7	12	10
8	10	9
9	9	9
10	9	11
11	11	12
12	12	11
13	11	10
14	10	12
15	12	12
16	12	8
17	8	11
18	11	8
19	8	10
20	10	11
21	11	11
22	11	9
23	9	8
24	8	9
25	9	10

The odd numbers rows represent the calibration set and other rows represent the validation set

Analysis of pharmaceutical preparation by the proposed methods

Appropriate volume of Flukanil® injection (75 mg/mL Rafoxanide) equivalent to 10 mg of rafoxanide was accurately taken and transferred into 100-mL volumetric flask and the volume was made up to 75 mL with methanol. The solution was shaken well then the volume was completed to 100-mL with the same solvent to obtain solution claimed to contain 100 µg/mL. Suitable dilutions were made using methanol to prepare aliquots covering the working concentration range. The spectra of these solutions were scanned from 225 to 415 nm and analyzed by the proposed methods.

RESULTS AND DISCUSSION

The zero-order absorption spectra of rafoxanide and its dgradate shows severe overlapping, as shown in **Figure 2**. The spectral overlapping of the drug and its degradation product prevents resolution of the mixture by the direct spectrophotometric measurements, Thus; we develop accurate and simple chemometric methods for determination of rafoxanide in presence of its degradate. The first step in model building, involves constructing the calibration matrix for rafoxanide and its degradate. In this study the model was optimized with the aid of the 5-level 2-factor design [10] resulting in 25 sample mixture. These 25 sample mixtures were divided to 13 training mixtures (odd numbers) for building the models and 12 validation mixtures (even numbers) for measuring predictive power of the models. The quality of multi component determination depends on the wavelength range and spectral mode used [11]. The wavelengths used were in the range 225-415 nm. Wavelengths less than 225 nm were rejected due to the noisy content. Wavelengths more than 415 nm were not used because they were uninformative (no absorption is mentioned in these regions). Cross-validation methods leaving out one sample at a time was employed [12]. The predicted concentrations were compared with the known concentrations of the compounds in each calibration sample. The root means squares error of cross-validation (RMSECV) was calculated for each method for examining the errors in the predicted concentrations. The selected model was that with the smallest number of factors such that RMSECV for that model was not significantly greater than RMSECV from the model with additional factor. Some factors were found to be optimum for the mixture of rafoxanide and degradate using PCR (**Figure 3**) and for PLS-1 (**Figure 4**). The percentage recoveries of the validation samples are shown in **Table 2** indicated the high predictive abilities of PCR, PLS and CLS models. The results obtained by applying the proposed methods compared to those obtained by applying the reported method [5]and no significant difference was observed(**Table 3**).

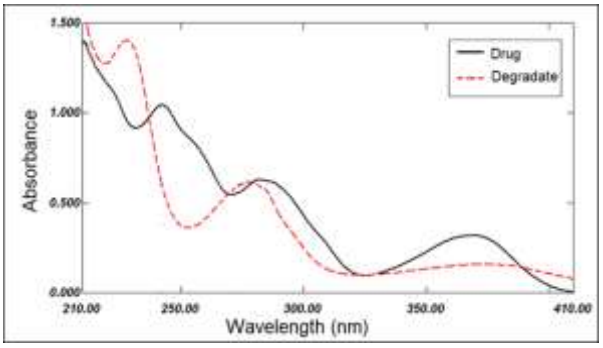

Fig. 2: Zero-order absorption spectra of rafoxanide (15 µg/ml) and its alkaline degradate (15 µg/ ml) (----).

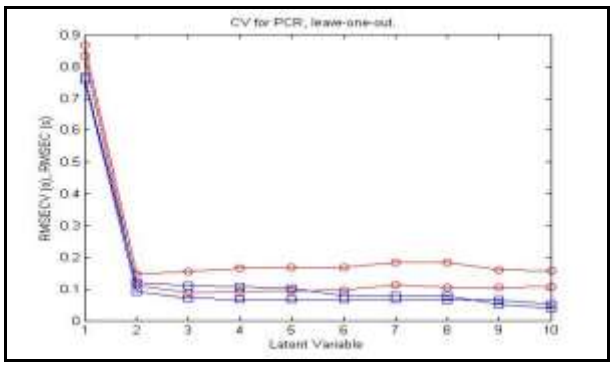

Fig. 3 : RMSECV plot of the cross validation results of the calibration set as a function of the number of latent variables (LVs) used to construct the PCR model

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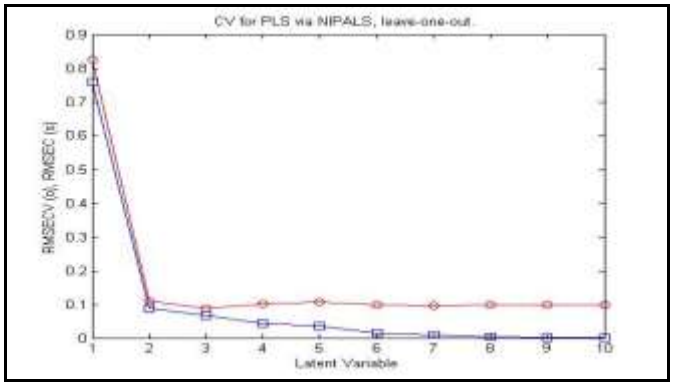

Fig. 4 : RMSECV plot of the cross validation results of the calibration set as a function of the number of latent variables (LVs) used to construct the PLS-1 model.

Table 2: Determination of rafoxanide and its degradate in validation set by the proposed chemometric methods.

Concentration (µg/ml)		CLS		PCR		PLS-1
Rafoxanide	degradate	Rafoxanide	degradate	Rafoxanide	degradate	Rafoxanide
10	8	98.58	100.31	98.51	100.44	98.52
8	12	97.94	98.72	98.11	98.51	98.13
9	12	100.05	98.25	99.06	98.08	97.96
10	9	98.81	98.29	98.77	98.35	98.80
9	11	98.47	99.55	98.56	99.41	98.59
12	11	98.27	99.67	98.66	99.70	99.51
10	12	99.47	99.74	98.85	99.62	99.55
12	8	100.54	102.91	100.43	103.19	100.43
11	8	100.10	100.51	100.00	100.73	100.00
10	11	99.72	99.66	99.75	99.60	99.75
11	9	100.53	100.78	100.46	100.91	100.46
8	9	99.26	99.79	99.31	99.71	99.28
Mean		99.31	99.85	99.21	99.85	99.25
RSD %		0.894	1.250	0.790	1.382	0.855
RMSEP*		0.107	0.115	0.105	0.127	0.101

*** Root mean square error of prediction.**

Table 3: Statistical comparison for the results obtained the proposed methods and reported method [5] for the analysis of rafoxanide in Flukanil® injection.

Parameters	CLS	PCR	PLS-1	Reported method
N[a]	5	5	5	5
$\bar{X}$[b]	100.04	99.91	99.88	100.37
SD	0.802	1.221	1.253	1.149
RSD%	0.802	1.222	1.255	1.145
t[c]	0.527 (2.31)	0.613 (2.31)	0.653 (2.31)	———
F[c]	2.052 (6.39)	1.128 (6.39)	1.189 (6.39)	———

[a]Number of experiments, [b]The mean of percent recovery of pharmaceutical preparation, [c] The values in parenthesis are tabulated values of "t "and "F" at (P = 0.05)

CONCLUSION

In conclusion, the described chemometric methods have the advantages of being simple, accurate and precise methods for determination of rafoxanide in bulk, pharmaceutical formulation and in the presence of its degradation product without sample pretreatment and without interference from excipients or degradate. The developed methods do not require sophisticated techniques or instruments and can be easily applied for quality analysis of the studied drug.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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