ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
29 (2002) 477–485

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Resolution of ternary mixtures of nitrofurantoin, furaltadone and furazolidone by partial least-square analysis to the spectrophotometric signals after photo-decomposition

M.C. Mahedero *, T. Galeano Díaz, S. Galán Pascual

Department of Analytical Chemistry, University of Extremadura, 06071 Badajoz, Spain

Received 3 August 2001; received in revised form 8 February 2002; accepted 24 February 2002

Abstract

An UV spectroscopic method is proposed to analyze mixtures of the nitrofuran derivatives, nitrofurantoin, furaltadone and furazolidone, used in veterinary. The change of absorption spectra due to photo-decomposition is used. A 20% dimethylformamide/water, basic medium of pH 9.4 (ammonium chloride/ammonia) and a time of irradiation of 15 s are selected. Calibration graphs are established, with the percentage of decrease of absorbance as analytical signal, in the range 2–10 μg ml^{-1}. To analyze mixtures of the three compounds the partial least-squares (PLS) multivariate analysis method is used with the spectra obtained by subtracting the spectra after irradiation to the original spectra. Good results have been obtained in the analysis of synthetic samples and a formulation containing all these compounds. © 2002 Published by Elsevier Science B.V.

Keywords: Nitrofurans; UV absorption spectroscopy; Photo-decomposition; Partial least-square multivariate analysis method

1. Introduction

Nitrofuran derivatives are highly effective chemotherapeutic drugs. Some of them are widely used to fight common infections in humans and animals or poultry. Among these compounds, nitrofurantoin (NF) (1-[[5-nitro-2-furanyl]methylene]amino-2,4-imidazolidinedione), furazolidone (FZ) (3-[5-nitrofurfurylideneamino]-2-oxazolidinone) and furaltadone (FD) ((5-morpholinomethyl-3-[5-nitrofurfurylideneamino]-2-oxazolidinone) are put together in a commercialized pharmaceutical product in Spain (Tribactina Premix from Esteve Lab [1]). This product has been of great use in the control of the intensive stock exploitation, being administered with feeds.

In the literature a great number of references about the spectrometric determination of nitrofurantoin or furazolidone have been found. In most of them, the use of different reagents to obtain a coloured product is described and the proposed methods are applied to the determination of these compounds in pharmaceutical preparations or biological fluids. However, few

* Corresponding author. Tel./fax: +34-924-289-375.
E-mail addresses: mahedero@unex.es (M.C. Mahedero), tgaleano@unex.es (T.G. Díaz).

0731-7085/02/$ - see front matter © 2002 Published by Elsevier Science B.V.
PII: S0731-7085(02)00092-4

references about the spectrometric determination of furaltadone appear in the literature [2–10].

On the one hand, the official AOAC method for determining furazolidone in finished feeds involved the colorimetric detection of the phenylhydrazine adduct [11].

A derivative spectrophotometric method has been proposed for determining NF in presence of FD and FZ [3]. This technique has also been used to determine these nitrofurans in presence of one or more different compounds in several formulations [8,12–14].

Other procedures like absorbance measures to diverse wavelengths and combination of the measured values [15–19] or multivariate analysis [9,10,20] have also been used. However, antecedents do not exist about the simultaneous determination of FZ and FD by spectrophotometry. This is explained by the great identity among their spectra of absorption and also among the changes that these experience in different media like, i.e. strongly alkaline solutions [4,5]. Chromatographic [21–24] methods has been proposed for their simultaneous determination, most of them in reversed phase. A voltammetric method to analyze the three compounds that makes use of a chemometric tool has also been described [25].

On the other hand it is well known that under the sunlight the nitrofurans solutions in a DMF:H_2O media are not stable [3,26].

In this paper the spectrophotometric behavior of the nitrofurans NF, FZ and FD under the irradiation UV is studied. Based on the differences between the final spectra, we propose a new method for determining these chemotherapeutic drugs in their mixtures. The multivariate PLS methods have been used in combination with the spectra obtained by subtracting the spectra after irradiation of solutions to those initially obtained. The proposed method allows the simultaneous determination of the three nitrofurans in study without need of carrying out previous separation. The advantages of this procedure respect to chromatographic methods is the use of very simple and cheap equipment and the solvent saving. And in comparison with the voltammetric method mentioned before, in our case we propose the use of the more easy and quick photometric technique.

2. Experimental

2.1. Apparatus

Spectronic 3000 diode-array Milton Roy photometer equipped with a PC 286 computer and Userdata v. 2.01 Milton Roy Inst. Co. software were use for spectra acquisition. Irradiation of samples was carried out with an Osram 200 W mercury arc lamp with an Oriel model 8500 power supply. The software packages Data Leader, v. 3.0, Beckman Instrument, and Grams 386 (Galactic Industries, USA) v. 3.04 with the PLSplus v. 2.1 application software, were used for the treatment of data.

2.2. Reagents

Furaltadone, furazolidone and nitrofurantoin obtained from Sigma were used. Standard solutions of these were prepared by dissolving of appropriate amount in dimethylformamide (DMF). NH_4Cl/NH_3 buffer solution (pH 9.4) was prepared from NH_4Cl and NH_3 provided by Panreac. All other chemicals were of analytical-reagent grade.

2.3. Pharmaceutical formulation

Tribactina Premix, composition per tablet: furaltadone hydrochloride, 2.4 mg; furazolidone, 4.8 mg; nitrofurantoin, 2.4 mg and exc.

2.4. General procedure for the nitrofurans determination

To determine the nitrofurans in Tribactina Premix, amounts of 50.0 mg of the veterinary product were weighed accurately and stirred carefully with 20 ml of DMF for 15 min. The residues were filtered and washed with a few milliliters of DMF, diluting the extracts to a final volume of 50 ml with DMF. Aliquots of 2.50 ml of these solutions were mixed, in 25 ml volumetric flasks, with 5 ml of 0.5 M NH_4Cl/NH_3 buffer solution (pH 9.4), 2.5 ml of DMF and deionized water to complete 25 ml. The absorption spectra of the samples are registered between 230 and 500 nm,

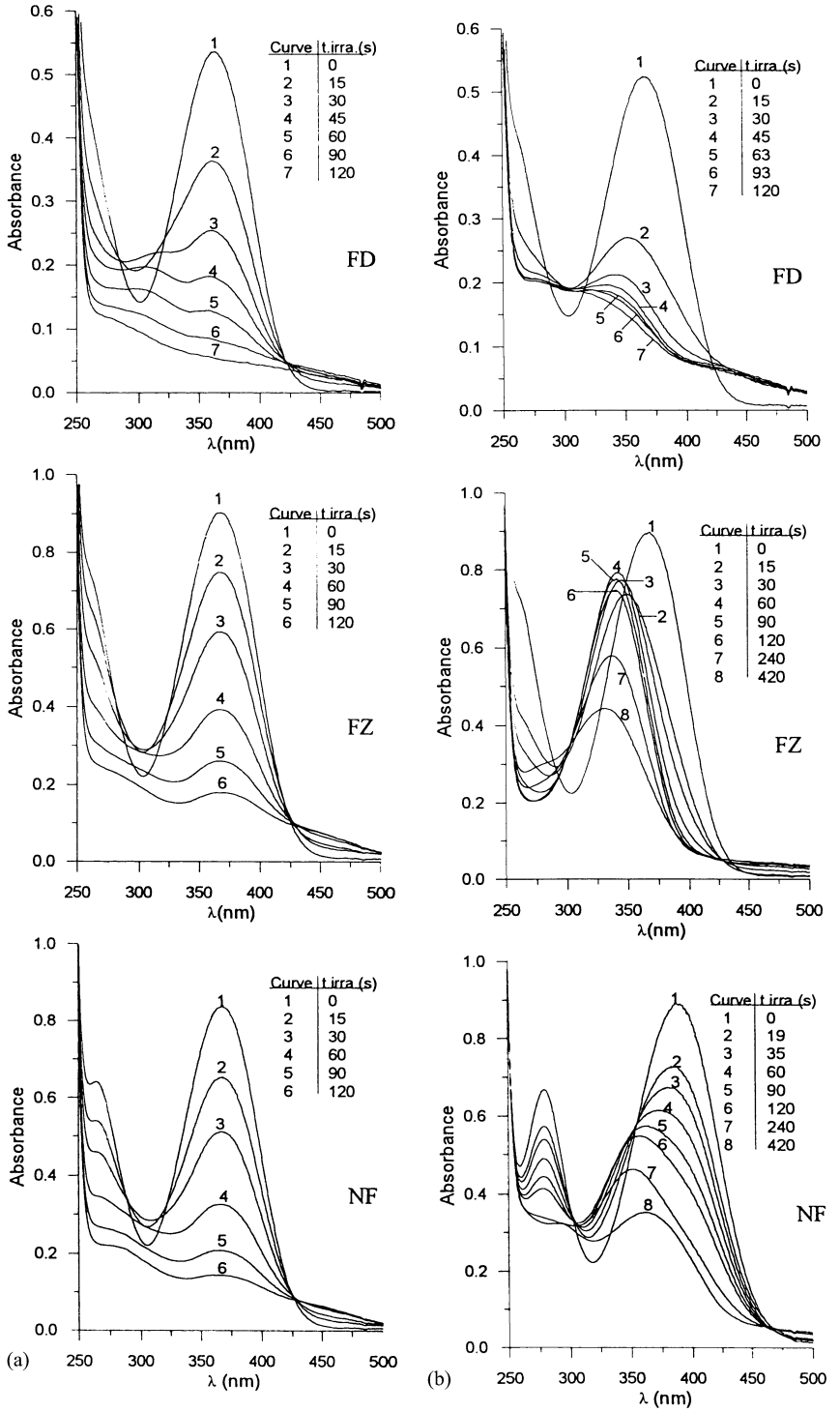

Fig. 1. Absorbance spectra of FD, FZ and NF for several irradiation times at pH 2.0 (a) and 9.4 (b).

against deionized water with 20% of DMF. The spectra are obtained before and after irradiating the solutions during 15 s and the difference between both were obtained and used as spectroscopic signals.

3. Results and discussion

As mentioned in the introduction, data about the light sensitive character of these nitrofurans are found in the bibliography. We have proved that when solutions containing 10 μg ml^{-1} of each nitrofuran in a DMF:H_2O (10:90) media, were exposed at sunlight for few minutes a slow decrease of the absorbance was observed according to the Refs. [3,26]. After 15 min of exposition a decrease of 10% was found. When these solutions are kept protected of the light, no additional changes were observed in their spectra of absorption for at least 120 min.

Then, the nitrofuran solutions were irradiated with UV light. The solutions placed in quartz cuvettes, were stirred and irradiated with the UV lamp during different periods. The changes observed in their absorption spectra are more important and depends on the pH value of solutions. Hence, in Fig. 1(a) and (b) the changes observed for 10 μg ml^{-1} solutions of the three nitrofurans in 10% DMF, at different pH, are shown. The change increases with the irradiation time. The decrease of absorbance at the wavelength of maximum ($A_{t=0} - A_{t=t_{irra}}$) is calculated in each case and expressed as percentage of the initial absorbance ($-\Delta A$ (%)) and the plot of this versus the irradiation time, in acid and basic media, is represented in Fig. 2.

The influence of pH on the change of spectra has been examined with solutions of the nitrofurans with pH values between 2 and 10 and registering the spectra after irradiating the solutions with different irradiation times (t_{irra}) In Fig. 3 the decrease of absorbance ($-\Delta A$ (%)), obtained with 30 s of irradiation, versus pH is shown.

The quickest decomposition takes place in strongly acid media and in basic media of pH 9.4 for the three compounds and also at around pH 5 for FD (Table 1). The FD is the most sensitive to the irradiation UV in all the studied media. After 1 min of irradiation the absorbance of FD solutions decreases near 80%.

In basic media, the decrease of the absorbance is also important, the maximum suffering displacements. In order to get the biggest difference between the spectra of FD and FZ with the irradiation, in subsequent experiences it was used a basic pH, by means of the addition of buffer solution 0.5 M NH_4Cl/NH_3 at pH 9.4.

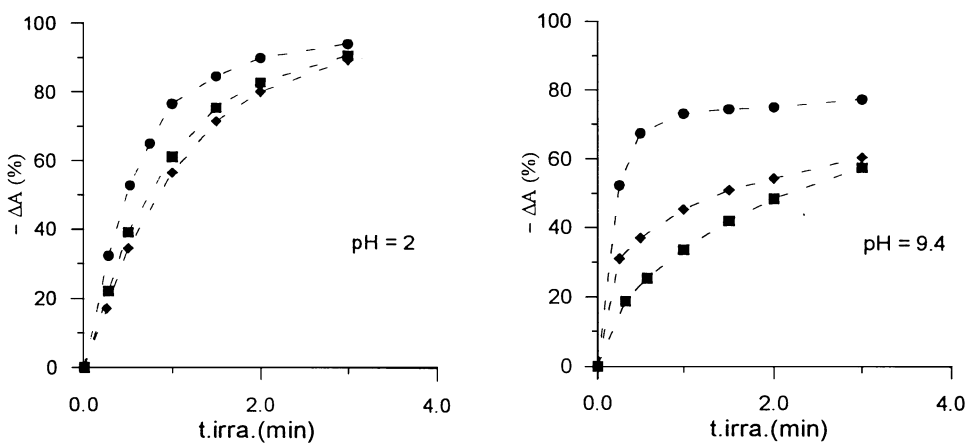

Fig. 2. Absorbance decrease in the function of the irradiation time with UV-light, ●, FD; ◆, FZ; ■, NF, $\lambda = 367$ nm.

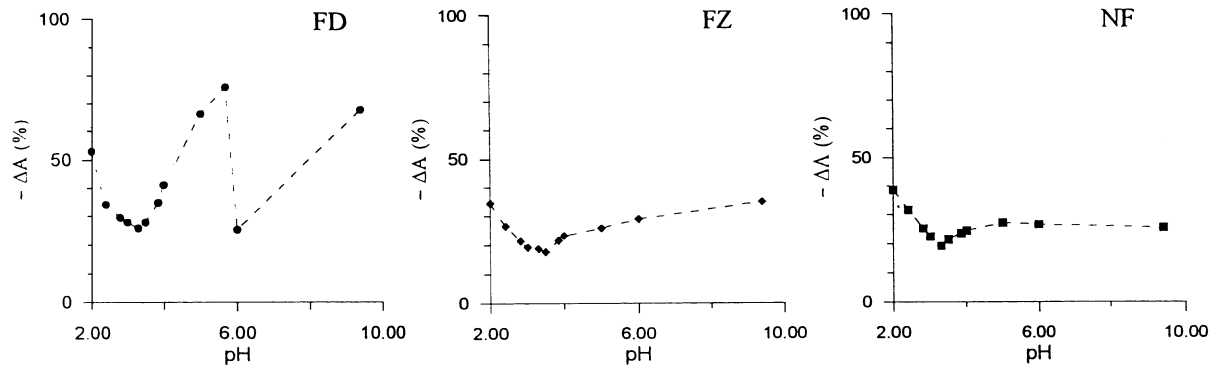

Fig. 3. Variation of the absorbance decrease for FD, FZ and NF, after 30 s of irradiation, in the function of pH.

Table 1
Decrease of absorbance ($\lambda = 367$ nm; $*\lambda = 388$ nm) with three irradiation times at different pH values

Compound	t_{irra} (s)	$-\Delta A$ (%)			
		PH 2.1	pH 5.2	pH 6.0	pH 9.4
FD	15	32.3	51.8	18.0	52.3
	30	52.8	66.2	25.2	67.4
	60	76.5	73.5	39.5	73.1
	15	17.2	22.9	22.0	31.0
FZ	30	34.4	25.8	28.4	36.9
	60	56.5	33.7	34.2	45.3
	15	22.2	23.7	25.3	14.7*
NF	30	39.1	27.2	26.6	23.3*
	60	61.1	34.1	32.5	33.6*

An irradiation time of 15 s was chosen as optimum. This time is enough for differenciate the three nitrofurans spectra. The irradiated solutions are stable if kept in the dark, at least 45 min.

The content of DMF does not affect in a significant way the decreased absorbance measured in the initial maximum, and we used the mixture DMF:H_2O (20:80) as solvent in later experiences.

3.1. Linearity of the signal

The calibration graph with the $-\Delta A$ (%) after 15 s of irradiation is obtained for each nitrofuran at pH 9.4. The linearity of this analytical signal was examined in the range 2.0–10.0 μg ml^{-1} of each one of these compounds. A good linearity is observed for the three compounds, being the statistical parameters in Table 2. The precision of the methods was checked by measuring replicate samples containing 5.0 μg ml^{-1} of each compound. The statistical parameters are given in Table 3.

3.2. Analysis of mixtures of nitrofurantoin, furaltadone and furazolidone

The difference among the spectrum of NF and the spectra of FD and FZ are enough to allow the determination of that in presence of these. However, the determination of mixtures of FD and FZ is not possible with spectroscopic methods even using a chemometric approach as differentiation of spectra [4,5], since their spectra are identical. However their behavior when irradiating is different, being some differences in the final spectra

obtained. In Fig. 4 the original spectra of FD and FZ are compared and also the spectra obtained after 15 s of irradiation of the solutions and the spectra obtained subtracting the previous (spectra subtraction). As can be seen the differences are greater in this last instance.

Table 2
Calibration graphs and statistical parameters for the $-\Delta A$ (%) of nitrofurantoin, furaltadone and furazolidone in alkaline medium (pH 9.4)

Compound	t_{irra} (s)	Equation	r^2	s_a	s_b	LOD_C	$LOD_{W\&L}$
NF ($\lambda = 388$ nm)	15	$Y = 1.398 \times 10^{-2} X + 1.223 \times 10^{-2}$	0.9744	4.167×10^{-3}	6.283×10^{-4}	1.43	0.90
	30	$Y = 1.667 \times 10^{-2} X + 1.360 \times 10^{-2}$	0.9869	3.554×10^{-3}	5.358×10^{-4}	1.02	0.64
	60	$Y = 2.033 \times 10^{-2} X + 1.920 \times 10^{-2}$	0.9873	4.239×10^{-3}	6.391×10^{-4}	1.00	0.63
FZ ($\lambda = 367$ nm)	15	$Y = 2.204 \times 10^{-2} X + 1.918 \times 0^{-3}$	0.9980	1.816×10^{-3}	2.738×10^{-4}	0.40	0.25
	30	$Y = 2.586 \times 10^{-2} X + 1.394 \times 10^{-2}$	0.9974	6.494×10^{-3}	9.789×10^{-4}	1.21	0.78
	60	$Y = 3.311 \times 10^{-2} X + 1.597 \times 10^{-2}$	0.9986	1.576×10^{-2}	2.376×10^{-3}	2.30	1.51
FD ($\lambda = 367$ nm)	15	$Y = 2.373 \times 10^{-2} X + 5.400 \times 10^{-3}$	0.9963	2.655×10^{-3}	4.002×10^{-4}	0.54	0.34
	30	$Y = 3.208 \times 10^{-2} X + 6.900 \times 10^{-3}$	0.9985	2.282×10^{-3}	3.441×10^{-4}	0.34	0.21
	60	$Y = 3.845 \times 10^{-2} X + 4.567 \times 10^{-3}$	0.9991	2.089×10^{-3}	3.149×10^{-4}	0.26	0.16

$Y = -\Delta A$ (%); X = concentration (μg ml^{-1}); LOD_C, calculated by the Clayton et al. method [29]; $LOD_{W\&L}$, calculated by the Winefordner and Long method [30]; s_a, intercept standard deviation; s_b, slope standard deviation.

Table 3
Statistical parameters of the repeatability of the absorbance decrease with 15 of irradiation

	Furaltadone ($\lambda = 367$ nm)	Furazolidone ($\lambda = 367$ nm)	Nitrofurantoin ($\lambda = 388$ nm)
Samples	10	12	12
$\bar{X}$	0.157	0.116	0.085
S_{N-1}	8.50×10^{-3}	4.08×10^{-3}	2.93×10^{-3}
RSD (%)	5.42	3.50	3.45

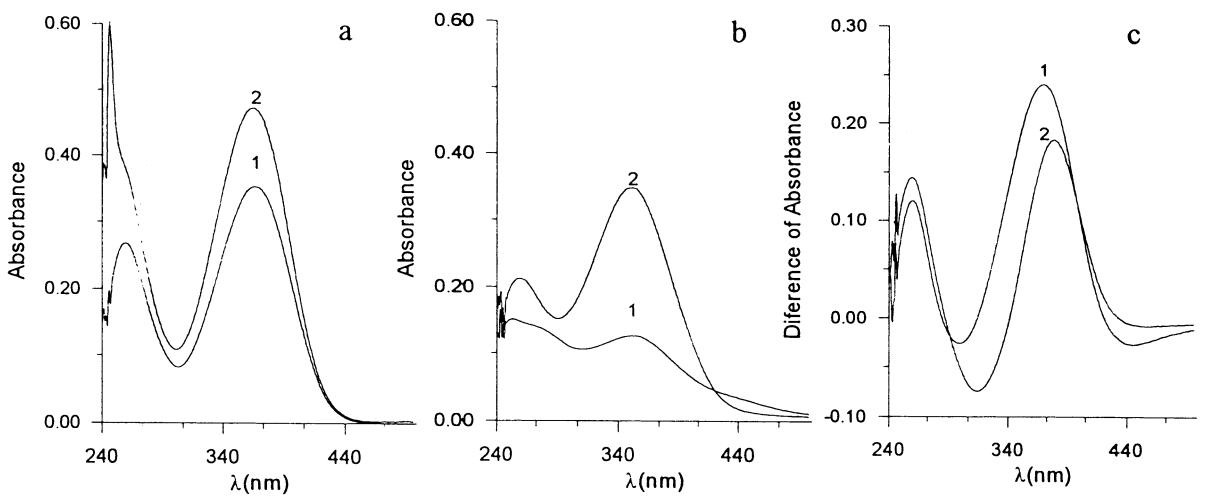

Fig. 4. (a) Original absorbance spectra; (b) absorbance spectra after irradiating 15 s; (c) subtraction of the previous spectra; (1) FD, (2) FZ; [FD] = [FZ] = 6 μg ml^{-1}.

Table 4
Composition of the samples of the calibration matrix for the PLS determination of nitrofurantoin–furazolidone–furaltadone

Sample	[FD] (μg ml^{-1})	[FZ] (μg ml^{-1})	[NF] (μg ml^{-1})	Sample	[FD] (μg ml^{-1})	[FZ] (μg ml^{-1})	[NF] (μg ml^{-1})
1	0	0	0	15	6	3	3
2	3	0	0	16	0	6	3
3	6	0	0	17	3	6	3
4	0	3	0	18	6	6	3
5	3	3	0	19	0	0	6
6	6	3	0	20	3	0	6
7	0	6	0	21	6	0	6
8	3	6	0	22	0	3	6
9	6	6	0	23	3	3	6
10	0	0	3	24	6	3	6
11	3	0	3	25	0	6	6
12	6	0	3	26	3	6	6
13	0	3	3	27	6	6	6
14	3	3	3				

Table 5
Statistical parameters with the application of PLS-1 to the different analytical signals

Compound	Number of factors	PRESS	SEP	RMSD	R^2
(a) Original spectra					
FD	9	2.0867	0.2833	0.278	0.98812
FZ	9	1.1189	0.2069	0.20303	0.99338
NF	4	0.0303	0.0341	0.0335	0.99982
(b) Spectra subtraction (difference between original spectra and spectra after irradiation)					
FD	5	1.4525	0.2364	0.23194	0.99104
FZ	8	0.7676	0.1718	0.16861	0.9953
NF	5	0.5677	0.1477	0.145	0.99651

Using this fact, calibration multivariate methods, PLS, were used for resolution of ternary mixtures of NF, FD and FZ. A matrix with 27 samples of well-known composition, following an orthogonal design, was prepared (Table 4). The results obtained using the absorption spectra and the spectra subtraction as analytical signals have been examined. To select the number of factors for PLS methods, the cross-validation methods, leaving out one sample at a time, has been used [27]. This process was repeated 27 times, until each sample had been left out once.

The prediction residual error sum of squares (PRESS) and the standard error of prediction (SEP) were calculated using a maximum number of factors of 15. The F statistic and the Haaland and Tomas criteria [28] were used to select the optimal number of factors through the comparison of PRESS values obtained using different number of factors.

In Table 5, the optimum number of factors, the PRESS values and statistical parameters obtained for all components by using the different signals, have been summarized.

The square of the correlation coefficient (R^2 of predicted concentration versus theoretical concentration of the analyte) is an indication of the quality of fit of all the data to a straight line in the optimized matrix. The values of the root mean square difference (RMSD) is an indication of the average error in the analysis for each component. The values of R^2 and RMSD are summarised in Table 5. It is observed that the values are slightly better for spectra subtraction.

The results obtained with different spectral regions have been studied. Also results by PLS-1 or PLS-2 have been studied. Significant differences do not exist.

3.3. Applications

The proposed methods and the optimized matrices have been used to determine the three nitrofurans in synthetic mixtures and in the veterinary formulation Tribactina Premix. In Table 6, the composition of these samples and the mean recoveries obtained are shown.

The obtained results using both matrices are similar when NF and FZ are determined. However, for FD best results are obtained using spectra subtraction.

The results obtained when the nitrofurans are determined in Tribactina Premix, are shown in Table 6, in reference with the values found by HPLC and with the labeled values. Better accordance can be appreciated, particularly in the case of the FD, when one makes use again of the spectra subtraction.

4. Conclusion

The different behavior of various nitrofurans, with regard to their photochemical degradation, allows us to propose a photometric method, which enables the multicomponent analysis of nitrofurantoin, furaltadone and furazolidone. This is a serious problem, particularly for the two last compounds, that are very similar. The PLS method proposed allows us to resolve the nitrofurans mixture. This method has been applied to the analysis of the nitrofurans in synthetic samples and in a veterinary formulation. The obtained results are similar to those obtained by HPLC, being the analysis very fast. Other advantage is the solvents saving.

Table 6
Composition of the synthetic problems and recoveries obtained for the PLS determination of nitrofurantoin–furazolidone–furaltadone using the calibration matrices of absorption spectra (1) and subtraction spectra (2)

Problem	Compound	Added (μg ml^{-1})	Recovery (%) (1)	Recovery (%) (2)
(a) Synthetic problems				
1	FD	2	96 ± 12.4	97 ± 18
	FZ	4	102 ± 6	109 ± 3
	NF	0	–	–
2	FD	4	90 ± 3	101 ± 4
	FZ	0	–	–
	NF	2	98 ± 4	112 ± 3
3	FD	0	–	–
	FZ	2	114 ± 51	105 ± 27
	NF	4	100 ± 3	107 ± 4
4	FD	4	97 ± 14	101 ± 2
	FZ	2	105 ± 26	105 ± 14
	NF	2	98 ± 4	109 ± 6
5	FD	2	92 ± 28	106 ± 3
	FZ	2	107 ± 28	94 ± 15
	NF	4	100 ± 1	104 ± 2
Compound	Labeled (mg g^{-1})	HPLC (mg g^{-1})	Found (mg g^{-1}) (1)	Found (mg g^{-1}) (2)
(b) Veterinary formulation: Tribactina Premix				
FD	41	37.3	25	39.8
FZ	120	124	125	117.5
NF	60	60.4	61	65

Acknowledgements

The authors gratefully acknowledge financial support from DGESIC of Spain (project BQU2000-0215). S. Galán Pascual gratefully acknowledges financial support from Fundación 'Fernando Valhondo Callaf' (Cáceres-Spain).

References

[1] A. Márques de Bonifaz (Ed.), Guía de productos zoosanitarios, Ed Veterindustria, Madrid, 1990.
[2] V.E. Egert, S.A. Giller, A.A. Lielzalve, M.V. Shimanskaya, Izv. Akad. Nauk Labv. SSR, Ser. Khim. (1963) 531–40.
[3] T. Galeano Díaz, L. López Martínez, F. Salinas, Analusis 22 (1994) 202–209.
[4] T. Galeano Díaz, L. López Martínez, F. Salinas, Mikrochim. Acta 112 (1993) 31–39.
[5] T. Galeano Díaz, L. López Martínez, F. Salinas, Microchem. J. 49 (1994) 61–68.
[6] P.L. Cox, J.P. Heotis, J. Agric. Food Chem. 11 (1963) 499–501.
[7] N.V. Kurinnaya, Farm. Zh. 27 (1972) 35–39 (C.A. 77:143846f).
[8] J.J. Berzas Nevado, J. Rodríguez Flores, M.L. Morena Pardo, Fresenius J. Anal. Chem. 349 (1994) 756–760.
[9] A. Espinosa Mansilla, F. Salinas, I. De Orbe Paya, Anal. Chim. Acta 313 (1995) 103–112.
[10] D. Ozdemir, R. Williams, Appl. Spectrosc. 53 (1999) 210–217.
[11] AOAC Official Methods of Analysis, Sydney Williams, 14th ed., 1984, 42.075, p. 797.
[12] J.J. Berzas Nevado, J. Rodríguez Flores, M.L. De la Morena Pardo, Analusis 21 (1993) 33–37.
[13] M.I. Walash, A.M. El Brashy, M.S. El Din, M.A. Abuirjeie, M.A. El Rahman Sultan, Pharmazie 49 (1994) 698–699.
[14] C.V.N. Prasad, V. Sripriya, R.N. Saha, P. Parimoo, J. Pharm. Biomed. Anal. 21 (1999) 961–968.
[15] S.M. Hassan, F. Belal, M.K.S. El-Din, M. Sultan, Anal. Lett. 21 (1988) 1199–1210.
[16] L. Elsayed, S.M. Hassan, K.M. Kelami, H.M. El-Fatatry, J. Assoc. Off. Anal. Chem. 63 (1980) 992–995.
[17] S. Wei, H. Yu, Z. He, Sichman Yixmeyuan Xuebao 15 (1984) 171–175 (C.A. 101:65464c).
[18] P.K. Chatterjee, C.L. Jain, P.D. Sethi, Indian J. Pharm. Sci. 48 (1986) 25–27.
[19] P.L. López de Alba, K. Wrobel, L. López Martínez, K. Wrobel, M.L. Yépez Murrieta, J. Amador Hernández, J. Pharm. Biomed. Anal. 16 (1997) 349–355.
[20] D. Basu, K.K. Mahalanabis, B. Roy, Anal. Chim. Acta 249 (1991) 349–352.
[21] N.M. Angelini, O.D. Rampini, H. Mugica, J. AOAC Int. 80 (1997) 481–485.
[22] T. Galeano Díaz, A. Guiberteau Cabanillas, M.I. Acedo Valenzuela, C.A. Correa, F. Salinas, J. Chromatogr. A 764 (1997) 243–248.
[23] S.M. Echterhoff, M. Petz, Dtsch. Lebensm. Rundsch. 90 (1994) 341–344.
[24] I. Kaniou, G. Zachariadis, G. Kalligas, H. Tsoukali, J. Stratis, J. Liq. Chromatogr. 17 (1994) 1385–1398.
[25] A. Guiberteau Cabanillas, T. Galeano Díaz, A. Espinosa Mansilla, F. Salinas, Talanta 41 (1994) 1821–1832.
[26] Leticia López Martínez, Tesis doctoral, Universidad de Extremadura, 1992.
[27] M.J.R. Stone, Stat. Soc. 36 (1974) 111.
[28] D.M. Haaland, E.V. Thomas, Anal. Chem. 60 (1988) 1193–1202.
[29] G.L. Long, J.D. Winefordner, Anal. Chem. 55 (1983) 712A–724A.
[30] C.A. Clayton, J.W. Hines, P.D. Elkins, Anal. Chem. 59 (1987) 2506–2514.