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Determination of dexamethasone and two excipients (creatinine and propylparaben) in injections by using UV-spectroscopy and multivariate calibrations

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Abstract

The use of multivariate spectrophotometric calibration for the simultaneous determination of dexamethasone and two typical excipients (creatinine and propylparaben) in injections is presented. The resolution of the three-component mixture in a matrix of excipients has been accomplished by using partial least-squares (PLS-1). Notwithstanding the elevated degree of spectral overlap, they have been rapidly and simultaneously determined with high accuracy and precision (comparable to the HPLC pharmacopeial method), with no interference, and without resorting to extraction procedures using non-aqueous solvents. A simple and fast method for wavelength selection in the calibration step is used, based on the minimisation of the predicted error sum of squares (PRESS) calculated as a function of a moving spectral window.

Keywords: Dexamethasone; Creatinine; Propylparaben; Multivariate calibration; Spectrophotometry

1. Introduction

Dexamethasone 21-phosphate [9α-Fluoro-16α-methyl-11β, 17α, 21-trihydroxy-1,4-pregnadiene-3,20-dione 21-phosphate] (DEX) is a synthetic glucocorticoid that is indicated for the treatment of several pathologies due to its anti-inflammatory and inmunosuppresor effects (Goodman-Hilman et al., 1996). It yields a symptomatic relief but it has no effects on the development of the underlying disease. When used in both, syrup and injection solutions, it is accompanied with excipients such as methyl and propylparaben (PRO), creatinine (CRE), sodium hydrogen sulphite, sodium citrate and sodium hydroxide (Martindale, 1993).

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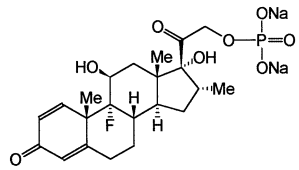
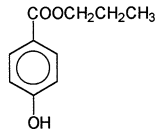
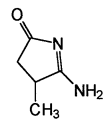


Dexamethasone 21-phosphate (disodium salt)



Propylparaben



Creatinine

Several methods are available for the determination of these drugs in syrups and other formulations: UV spectrophotometry (Chen et al., 1991), differential UV spectrophotometry (Xi et al., 1991), HPLC (Gagne and Lodge, 1980; Das-Gupta, 1979), reverse-phase HPLC (Yao and Qiao, 1988) and TLC (Datta and Das, 1993; Das et al., 1986).

The determination of DEX by conventional spectrophotometry without prior separation procedures is not possible due to the overlapping of dexamethasone and excipients UV spectra. However, multivariate calibration methods applied to both absorptive and emissive spectral data are being increasingly used for the analysis of complex pharmaceuticals mixtures. These methods show the advantage of using full spectral information, and allow for a rapid determination of mixture components, often with no need of prior separation or sample pre-treatment. We have recently reported the simultaneous determination of complex pharmaceutical mixtures as antibiotics in tablet formulations (Goicoechea and Olivieri, 1999a), a cold-cough syrup of bromhexine (Goicoechea and Olivieri, 1999b), an ophthalmic solution of timolol and pilocarpine (Satuf et al., 1999), teophylline (Goicoechea et al., 1999) and tetracycline both in serum (Goicoechea and Olivieri, 1999c), by partial least-squares (PLS) regression using the PLS-1 formalism and the recently introduced hybrid linear analysis (HLA). An advantage of robust multivariate methods, such as PLS and HLA, is that calibration can be performed by ignoring the concentrations of all other components except the analyte of interest. This makes these methods especially appealing for the determination of the active components in ophthalmic solutions, syrups and injections whose excipients may show absorption spectra, which are severely overlapped with those from the analytes.

In this report, we discuss the simultaneous determination of dexamethasone, creatinine and propylparaben in injection by UV-spectrophotometry and the popular multivariate calibration tool: PLS-1.

2. Experimental

2.1. Apparatus

Electronic absorption measurements were carried out on a Perkin–Elmer Lambda 20 spectrophotometer, using 1.00 cm quartz cells. All spectra were saved in ASCII format, and transferred to a PC Pentium 550 microcomputer for subsequent manipulation. PLS-1 was applied with the program MULTIVAR (Goicoechea and Olivieri, 2000) and available at fttp://fbioyf.unr.edu.ar/Cientifica/multivar.exe. The Sigmaplot 5.0 software was used for regression analysis and treatment of data. Wavelength selection was carried out by a moving-window-minimum PRESS strategy (Collado et al., 2000) implemented with the program MULTIVAR.

2.2. Reagents

All experiments were performed with analytical-reagent grade chemicals. Stock solutions of dexamethasone disodium phosphate (600.00 μg ml^{-1}), creatinine (800.00 μg ml^{-1}) and propylparaben (300.00 μg ml^{-1}) were prepared by dissolving the compounds in doubly distilled water. A mixture of the others excipients was prepared in distilled water: sodium hydrogen sulphite 2.02 mg ml^{-1}, sodium citrate 1.13 mg ml^{-1} and sodium hydroxide 1.60 mg ml^{-1}.

2.3. PLS Calibration set

In the presently studied solutions, in which the analyte of interest is embedded in a complex mixture of a large number of components, a training set of 15 samples with a central composite design was prepared for calibration, with

the concentrations of dexamethasone lying in the known linear absorbance-concentration range (see Table 1). These samples were prepared by dilution of a convenient amount of the stock solutions. The levels of the components in the mixtures were chosen in order to include a range of 80–120% of the amounts present in the commercial samples as recommended by regulatory agencies (Green, 1996).

2.4. Validation set number one

One validation set was built with 45 samples containing three different levels of the studied compound, also containing a level of excipients corresponding to 100% of the amount labelled by the manufacturer laboratory. These samples were prepared by dilution of a convenient amount of the stock solutions. The active ingredient was present in concentrations which were similar to a commercially available sample $\pm 20\%$ (Table 1). Three groups of 15 samples were prepared and analysed in 3 consecutive weeks. This procedure allowed us to assess both inter- and intra-assay precision and accuracy.

2.5. Validation set number two

A set of 15 samples with concentrations from 50 to 150% of the amounts of DEX which are usually present in the commercial samples was built and used to obtain the recovery (%) and to

Table 1
Composition of both calibration set (using a central composite design) and validation set number one for applying PLS-1 method

Calibration and validation samples	DEX (mg l^{-1})	CRE (mg l^{-1})	PRO (mg l^{-1})	Excipients (%)[a]
C1	2.50	4.00	0.85	100
C2	2.50	4.00	3.20	100
C3	2.50	15.00	0.85	100
C4	2.50	15.00	3.20	100
C5	9.50	4.00	0.85	100
C6	9.50	4.00	3.20	100
C7	9.50	15.00	0.85	100
C8	9.50	15.00	3.20	100
C9	0.00	9.50	2.00	100
C10	12.00	9.50	2.00	100
C11	6.00	0.00	2.00	100
C12	6.00	19.20	2.00	100
C13	6.00	9.50	0.00	100
C14	6.00	9.50	4.00	100
C15	6.00	9.50	2.00	100
V1	6.40	12.80	2.70	100
V2	6.40	12.80	2.70	100
V3	6.40	12.80	2.70	100
V4	6.40	12.80	2.70	100
V5	6.40	12.80	2.70	100
V6	8.00	12.80	2.70	100
V7	8.00	12.80	2.70	100
V8	8.00	12.80	2.70	100
V9	8.00	12.80	2.70	100
V10	8.00	12.80	2.70	100
V11	9.60	12.80	2.70	100
V12	9.60	12.80	2.70	100
V13	9.60	12.80	2.70	100
V14	9.60	12.80	2.70	100
V15	9.60	12.80	2.70	100

[a] The % is relative to a usual commercial injection blank.

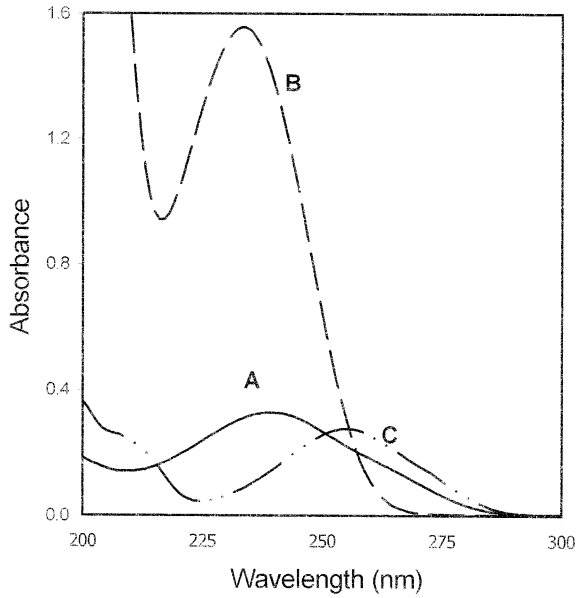


Fig. 1. Electronic absorption spectra in aqueous solution for: (A) DEX (8.0 mg l^{-1}), (B) CRE (12.8 mg l^{-1}) and (C) PRO (2.7 mg l^{-1}).

construct plots of predicted versus actual concentrations for the joint confidence test for the slope and the intercept (see Table 5 and Fig. 2). These samples were undergone to the same procedure that the commercial ones.

2.6. Commercial sample

One commercial sample was tested: Dexamethasone Larjan (Veinfar Laboratories, Argentine), an injection containing (per ml) 4.0 mg of dexamethasone hydrogen phosphate, creatinine 6.4 mg and propylparaben 1.4 mg. The sample was prepared by diluting 1.00 ml of the solution with doubly distilled water in a 500.00 ml volumetric flask before measurements.

3. Results and discussion

Fig. 1 shows the spectra of DEX, PRO and CRE in aqueous solution. As can be seen, the strong overlapping of the components hinders the resolution of the mixture by conventional spectrophotometry. A usual and popular method for resolving mixtures, which can be applied to the present case, is partial least squares analysis (PLS).

Electronic absorption spectra for the standard samples shown in Fig. 1 were recorded in the range 200–350 nm and subjected to PLS-1 analysis. The optimum number of factors to be used within the PLS-1 algorithm is an important parameter to achieve better performance in prediction. This allows us to model the system with the optimum amount of information, avoiding overfitting. The cross-validation procedure was applied, consisting of systematically removing one of the training samples in turn, and using only the remaining ones for construction of the latent factors and regression (Thomas and Haaland, 1988). Table 2 gives the optimum number of factors, the values of the optimal regions used and statistical parameters. Wavelength selection is a critical step for increasing the predictive ability of multivariate analysis, and should ideally eliminate both uninformative and/or highly correlated data. In a previous report we used a moving window strategy in order to eliminate interferences by selecting the optimal spectral region for a specific sample (Goicoechea and Olivieri, 1999d). In the present report we have applied a moving window strategy to the calibration set itself, in order to find the

Table 2
Optimum number of factors and calibration statistical parameters when applying PLS-1 analysis

Statistical parameters[a]	DEX	CRE	PRO
Spectral region (nm)	220–320	240–280	260–340
Factors	4	4	4
PRESS (mg $l^{-1} \times 10^5)^2$	0.0796	0.0716	0.0242
RMSD (mg $l^{-1} \times 10^5$)	0.075	0.0691	0.0402
REP%	1.311	0.97	0.97
r^2	0.9995	0.9998	0.9997

[a] $\text{PRESS} = \Sigma_1^I (c_{\text{act}} - c_{\text{pred}})^2$, $\text{RMSD} = \left[1/I\Sigma_1^I (c_{\text{act}} - c_{\text{pred}})^2 \right]^{1/2}$, $\text{REP\%} = 100/c \left[1/I\Sigma_1^I (c_{\text{act}} - c_{\text{pred}})^2 \right]^{1/2}$ and $r^2 = 1 - \Sigma_1^I (c_{\text{act}} - c_{\text{pred}})^2 / \Sigma_1^I (c_{\text{act}} - \bar{c})^2$, $\bar{c}$ is the average component concentration in the I calibration mixtures.

Table 3
Validation results for dexamethasone when applying PLS-1 analysis

Validation samples	Added (mg l^{-1})	Week 1		Week 2		Week 3	
		Found (mg l^{-1})	Recovery (%)	Found (mg l^{-1})	Recovery (%)	Found (mg l^{-1})	Recovery (%)
DEX							
1	6.40	6.40	100.0	6.36	99.4	6.57	102.7
2	6.40	6.59	103.0	6.54	102.2	6.53	102.0
3	6.40	6.51	101.7	6.51	101.7	6.58	102.8
4	6.40	6.56	102.5	6.57	102.6	6.57	102.7
5	6.40	6.57	102.6	6.47	101.1	6.60	103.1
6	8.00	8.03	100.4	8.19	102.4	8.31	103.9
7	8.00	8.08	101.0	8.12	101.5	8.22	102.7
8	8.00	8.11	101.4	8.15	101.9	8.25	103.1
9	8.00	8.05	100.6	8.21	102.6	8.30	103.7
10	8.00	8.10	101.2	8.20	102.5	8.18	102.2
11	9.60	9.64	100.4	9.79	102.0	9.91	103.2
12	9.60	9.68	100.8	9.78	101.9	9.87	102.8
13	9.60	9.65	100.5	9.83	102.4	9.88	102.9
14	9.60	9.75	101.6	9.89	103.0	9.80	102.1
15	9.60	9.66	100.6	9.88	102.9	9.92	103.3

most informative range in the spectra by localisation of the minimum PRESS (Collado et al., 2000; Luis et al., 2001). Table 2 shows the optimal spectral ranges found by using the above method.

The optimal PLS-1 calibration was applied to the prediction of the concentrations of the components in both real and synthetic samples, with the results collected in Tables 3–5. Tables 3 and 4 show the results obtained for DEX and the two excipients (CRE and PRO), respectively, in the validation set number one, applying the PLS-1 method. The analyses were performed in three consecutives weeks, in order to evaluate inter- and intra-assay accuracy and precision. As regards the results provided by PLS-1 on the validation set number one, very good recoveries were obtain for DEX, CRE and PRO (with average recovery % of 102.0, 101.4, 104.3, respectively).

Table 5 shows the results obtained for DEX in the validation set number two. As can be seen, the recoveries were excellent in all the studied levels. With these samples, the plot of c_{pred} versus c_{act} was constructed. The results are: slope = 1.03 (1) and intercept = -0.10 (6). Conventional individual confidence intervals for the slope and the intercept can lead to erroneous conclusions when carried out independently of each other, since this ignores their strong mutual correlation. Instead of these individual tests, the elliptic joint confidence region (EJCR) for the slope and intercept is recommended (González et al., 1999). Fig. 2 shows that region for DEX. It contains the theoretically expected value of (1,0). It can thus be concluded that bias is absent for the determination of DEX when using PLS-1. Table 5 also shows the result obtained when analyzing DEX on a commercial sample, Dexamethasone Larjan (Veinfar Laboratories, Argentine). The same sample was analysed by HPLC (XXIV United States Pharmacopeia, 2000) with a comparable result (recovery of 99.5%).

4. Conclusions

The contents of dexamethasone and two excipients (creatinine and propylparaben) in injections were simultaneously determined using electronic absorption measurements, together with PLS-1 multivariate calibration analysis. A validation set

Table 4
Validation results for creatinine and propylparaben when applying PLS-1 analysis

	CRE			PRO		
	Week 1	Week 2	Week 3	Week 1	Week 2	Week 3
Added (mg l^{-1})	12.8	12.8	12.8	5.4	5.4	5.4
Found[a] (mg l^{-1})	12.87 (0.06)	13.07 (0.03)	13.01 (0.03)	5.51 (0.05)	5.72 (0.07)	5.67 (0.05)
Recovery % average intra-assay	100.5	102.1	101.7	102.1	106.0	105.0
V.C. % intra-assay	0.50	0.31	0.24	0.97	1.17	0.94

[a] Values in parenthesis correspond to standard deviations ($n = 15$).

Table 5
Results obtained when applying PLS-1 analysis for DEX on both artificial and commercial samples

Samples[a]	DEX (mg ml^{-1})		
	Added	Found	Recovery (%)
1	9.05	9.37	103.5
2	8.40	8.49	101.1
3	7.87	8.01	101.8
4	7.24	7.49	103.4
5	6.97	7.10	101.9
6	6.61	6.67	100.9
7	6.34	6.50	102.5
8	6.06	6.01	99.2
9	5.70	5.68	99.6
10	5.43	5.46	100.5
11	5.16	5.11	99.0
12	4.80	4.77	99.4
13	4.25	4.23	99.5
14	3.62	3.64	100.5
15	2.99	3.04	101.7
Larjan[b]	4.00	4.01	100.3

[a] The samples were diluted (1:500) before analysis. Five replicates were analysed.

[b] The recovery obtained by HPLC (USP XXIV, 2000) was 99.5%.

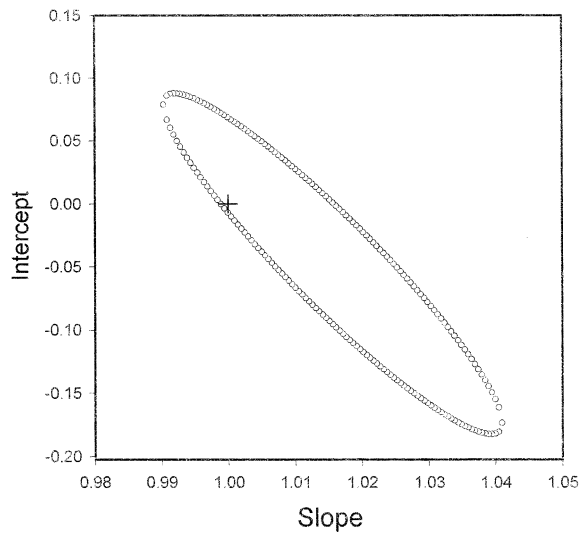


Fig. 2. Elliptic joint confidence region for the slope and intercept for the validation data set number 2 (including 15 values), as calculated from determination of DEX by using PLS-1 analysis. The cross marks the theoretical point (1,0).

of synthetic mixtures for accuracy and precision, as well as a commercial pharmaceutical were studied. Excellent recoveries and coefficients of variations were obtained in all cases. The elliptic joint confidence region (EJCR) for the slope and intercept showed that the bias is absent for the determination of DEX. The substantial reduction of analysis time, which is achieved with the present method in comparison with HPLC makes the former one suitable for control analyses of the injection studied.

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