SHORT COMMUNICATION

Patricia M. Castellano · Silvana E. Vignaduzzo
Rubén M. Maggio · Teodoro S. Kaufman

Application of a chemometric method for simultaneous determination of acetaminophen and diclofenac in content-uniformity and drug-dissolution studies

Abstract A new, repeatable, and rapid method has been developed for resolution of binary mixtures of acetaminophen and diclofenac with minimum sample pretreatment and without separation of the analytes. The method, based on the PLS1 processing of absorbance data in the UV region, was successfully used for quantification of the drug content of three tablet preparations. The results obtained were in good agreement with HPLC recovery data. The method also enabled determination of drug-dissolution profiles of these commercial tablets, by simultaneous determination of both analytes during the dissolution test.

Keywords Chemometric method ·
Acetaminophen · Diclofenac · PLS · Dissolution

Introduction

Acetaminophen (ACE) and diclofenac (DIC) are a successful therapeutic combination, very useful in many specific conditions [1–4]. The widespread use of this pharmaceutical association has stimulated the development of analytical methods for simultaneous determination of both components. Such methods include spectrophotometry [5], HPTLC [6], HPLC [7–9], GC [10], supercritical-fluid chromatography [11], and MEK capillary electrophoresis [12]. A solid-phase spectro-

P. M. Castellano · S. E. Vignaduzzo · R. M. Maggio
T. S. Kaufman
Area Análisis de Medicamentos, Facultad de Ciencias Bioquímicas
y Farmacéuticas, Universidad Nacional de Rosario,
Suipacha 531, Rosario (S2002LRK), Argentina

T. S. Kaufman (✉)
Instituto de Química Orgánica de
Síntesis -IQUIOS- (CONICET-UNR), Suipacha 531,
Rosario (S2002LRK), Argentina
E-mail: tkaufman@fbioyf.unr.edu.ar
Tel.: +54-341-4804592
Fax: +54-341-4370477

photometric method capable of determining DIC in the presence of a fivefold excess of ACE has also been reported [13]. It is noteworthy that only one chemometrics-assisted method for the quantification of both drugs, employing artificial neural networks, has yet been reported [14]; this method has, however, been studied only on synthetic mixtures and with drug ratios different from those frequently used in human therapeutics.

We report herein the development of a chemometric method, suitable for simultaneous determination of ACE and DIC in synthetic samples and pharmaceutical formulations, based on the PLS1 (partial least squares algorithm with one dependent variable) analysis of sample spectral data in selected regions of the ultraviolet region. The method is simple, fast, accurate, highly suitable for routine quality-control determinations, and useful for analysis of drug content in combined tablet formulations and for the determination of drug-dissolution profiles for these preparations.
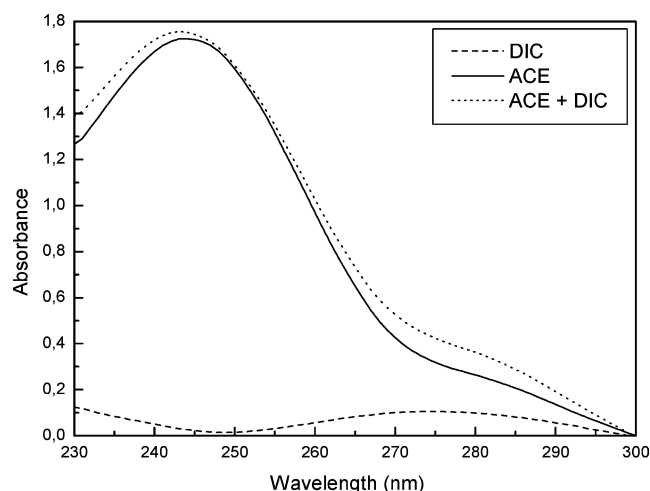
Results and discussion

ACE and DIC are usually marketed as a combined formulation with a fixed ACE/DIC ratio of 6:1 (w/w), although other proportions are also available. Figure 1 shows the electronic excitation spectra of ACE, DIC, and a 6:1 (w/w) mixture of both drugs in the 230–300 nm region. Because of the unfavourable concentration relationship of DIC and because of its comparatively poor specific absorption, the absorbance of the latter is very low compared with that of ACE. Furthermore, observation of the spectra of the drugs clearly shows they overlap severely, hindering their simultaneous determination by classical methodology because of mutual interference. This prompted other authors to use differential extraction, readings at several wavelengths, and mathematical calculations to alleviate the interference and overcome the comparatively low absorption of DIC.

Multivariate calibration methods of analysis such as PLS1 are gaining wide approval for resolution of mix-

Fig. 1 Ultraviolet spectra of the analytes between 230 nm and 300 nm: *continuous line*, ACE, 27.0 mg L^{-1}; *dashed line*, DIC, 4.5 mg L^{-1}, and *dotted line*, mixture of ACE (27.0 mg L^{-1}) and DIC (4.5 mg L^{-1}) in 10^{-4} mol L^{-1} HCl

tures of compounds of pharmaceutically interest [15]; they are also continuously finding new analytical uses [16]. We therefore expected that application of this chemometric algorithm [17, 18] to sample spectra, could overcome the spectral overlapping problem and be of help for simultaneous determination of both drugs while retaining good accuracy and precision for quantifying the less-absorbing species.

To develop our method two calibration models were built, one with a narrow concentration range of analytes, useful for drug content analysis, and the other with a broader concentration range, to be employed for the determination of dissolution profiles. Each of the calibration models was internally validated with 16 mean-centred spectra of samples, conforming to four-level full-factorial designs and having known con-

tents of ACE and DIC [19]. For improved results, wavelength interval selection was made, employing the variable-size moving-window strategy [20]. Critical data of the calibration are shown in Table 1.

The quality of the models was inferred from values of the squares of the correlation coefficients (r^2), the relative errors of prediction during calibration (REC), and the analytical figures of merit. Furthermore, the models were externally validated and the stability of the calibrations was tested with three external validation sets of six synthetic samples each, analysed on three separate occasions after internal validation of the calibration models. Overall, mean recovery values for the drug content analysis model were close to quantitative ($100.1 \pm 0.4\%$ for ACE and $97.9 \pm 0.8\%$ for DIC, 18 determinations each) and the method was shown, by use of ANOVA tests, to be precise and repeatable.

The method was used for analysis of six synthetic samples, furnishing analyte recoveries of $100.2 \pm 0.3\%$ for ACE and $99.3 \pm 0.8\%$ for DIC. Next, it was applied to the determination of ACE and DIC in three commercial pharmaceutical tablet preparations. To comply with the stated content of the active principles is a legal requirement and, therefore, drug content is one of the most important properties describing the quality of tablets. As shown in Table 2, tablets of all the tested brands were shown to comply with the declared amounts of their pharmacologically active drugs. Results were supported by the very good standard deviation and relative standard deviation values ($< 2\%$) observed.

Thus, although data dispersion for determinations of DIC, the less concentrated analyte, was slightly higher than those for ACE, the precision of the determinations was always very good. Moreover, when the samples were analysed by HPLC, following the USP 26 conditions, as a reference method [21] no statistically significant differences were observed.

Table 1 Statistical data for the calibration models for the UV–PLS1 simultaneous analysis of acetaminophen and diclofenac

Property of interest[a]	ACE		DIC	
Analytical purpose	Drug content	Dissolution[b]	Drug content	Dissolution[b]
Optimum spectral range (nm)	259–300	289–299	264–296	280–299
Number of sensors	42	11	33	20
Concentration range (mg L^{-1})	19.97–29.95	27.2–68.0	2.94–4.42	4.0–10.0
Number of calibration samples	16	16	16	16
Number of PLS factors	2	2	4	4
PRESS [(mg L^{-1})^2]	0.24	1.77	0.058	0.12
RMSD (mg L^{-1})	0.40	0.72	1.09	0.80
REC (%)	0.45	0.70	1.12	0.79
r^2	0.9993	0.9996	0.9966	0.9996
Selectivity	0.52	0.14	0.31	0.056
Sensitivity (SEN)	0.06	0.025	0.013	0.0063
Analytical sensitivity [(γ), L mg^{-1}]	3.03	0.52	5.31	1.39
Minimum concentration difference [(γ^{-1}), mg L^{-1}]	0.33	1.91	0.19	0.72

[a]PRESS $= \Sigma(C_{pred}-C_{act})^2$, RMSD $= [1/N\Sigma(C_{pred}-C_{act})^2]^{1/2}$, REC (%) $= (100/C_{mean})$ $[1/N\Sigma(C_{pred}-C_{act})^2]^{1/2}$, and $r^2 = 1-[\Sigma(C_{pred}-C_{act})^2/\Sigma(C_{act}-C_{mean})^2]$, where C_{mean} is the average component concentration in the N calibration mixtures. Sensitivity $= 1/||b_k||$, where b_k is the final regression coefficients vector for component k, and $\gamma = (SEN/\sigma_0)$, where σ_0 is the standard deviation of the blank. Selectivity $= 1/(||b_k||$ $||AC/C^TC||)$, where A and C are the mean-centred absorbance (within the region of interest) and concentration data blocks, respectively.
[b]In 10^{-4} mol L^{-1} HCl

Table 2 Simultaneous UV-PLS1 determination of acetaminophen and diclofenac in three commercial tablet formulations

Drug			ACE[b]			DIC[b]		
Method	Data[a]		Brand 1	Brand 2	Brand 3	Brand 1	Brand 2	Brand 3
UV-PLS1	Mean recovery (%)		99.2	99.7	100.5	106.9	107.2	100.7
	Mean recovery (mg/tablet)		297.6	299.1	301.5	53.5	53.6	50.4
	SD (mg/tablet)		3.6	3.0	2.1	0.7	0.8	0.9
	RSD (%)		1.2	1.0	0.7	1.4	1.6	1.8
	Confidence limit[c]		2.9	2.4	1.7	0.6	0.7	0.7
HPLC	Mean recovery (%)		98.9	98.2	98.9	106.4	107.6	100.0
	RSD (%)		1.6	0.6	1.5	0.6	0.2	1.2
	$t_{(calc.)}^{d}$		0.37	3.12	2.31	1.12	1.21	0.79

[a]For six replicates, SD: standard deviation, RSD: relative standard deviation
[b]Tablet label declared content to be 300 mg ACE and 50 mg DIC

[c]Expressed in mg/tablet. $P = 0.05$
[d]$t_{(5, 0.01)} = 3.17$

Chemometric methods have recently been recognised as valuable means of determination of dissolution profiles, because they enable high sample throughput [22–24]. The profiles are not only valid for measuring the availability of the active ingredients but also constitute useful means of testing the reproducibility of the manufacturing process. We therefore used the proposed method to construct dissolution profiles of ACE and DIC in their combined tablet formulations.

The broad concentration model was employed (Table 1) to improve detection of small amounts of both drugs, especially DIC, characteristic of the first stages of dissolution. As expected, although statistical indicators of the calibration models were regarded as satisfactory, because of its broad range, selectivity, sensitivity, and minimum detectable concentration were slightly lower than for the optimised model used for assay of drug content.

The dissolution step of the test was implemented in 10^{-4} mol L^{-1} HCl, in accordance with USP guidelines with regard to media volume, apparatus type, and stirring rate [21]. After several preliminary tests conducted to optimise sample dilution and processing, the three commercial formulations were examined. The results are depicted in Fig. 2. Data dispersion among the vessels was considered acceptable, with RSD values slightly higher (6–8%) in the climbing part of the profiles than during the last stages (2–4%). These observations, which agreed with HPLC results, were attributed to small time differences during sample withdrawal and to known manufacture-related differences between the tablets in this test.

In conclusion, a new analytical method was developed for simultaneous determination of ACE and DIC in synthetic samples and pharmaceutical dosage forms. Under appropriate conditions the proposed procedure, based on the PLS1 analysis of UV spectra, enabled simultaneous determination of the amounts of the active principles in synthetic binary mixtures and commercial tablets containing both ACE and DIC. The method also enabled acquisition of the dissolution profiles for each of this pair of co-formulated drugs, the spectra of which overlapped severely, under the dissolution guidelines of USP 26. The proposed method requires minimum sample pre-treatment and uses commonly available equipment and reagents. Apart from these valuable merits, it is a rapid, accurate, and convenient alternative for simultaneous determination of ACE and DIC in the

Fig. 2 Dissolution profiles of ACE (*left*) and DIC (*right*) for three combined commercial formulations, as determined by the UV-PLS1 method
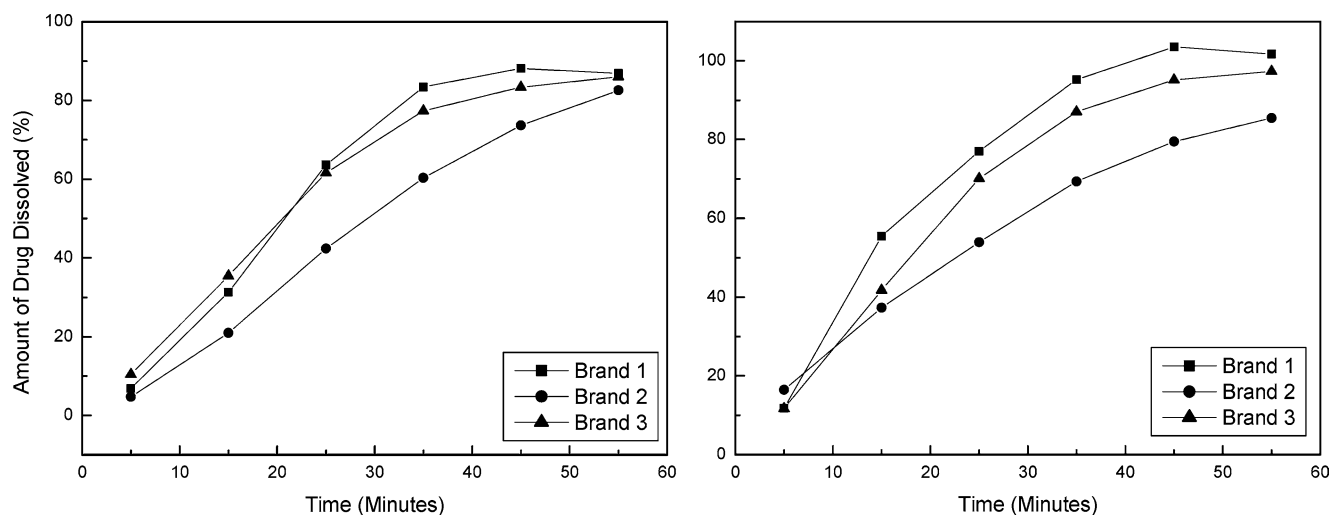
routine quality control of their combined pharmaceutical formulations.

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