Simultaneous Determination of Dapsone and Pyrimethamine by Derivative Spectrophotometry in Pharmaceutical Formulations

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A simple and fast method was developed for the simultaneous determination of dapsone and pyrimethamine by first-order digital derivative spectrophotometry. Acetonitrile was used as a solvent to extract the drugs from the pharmaceutical formulations, and the samples were subsequently evaluated directly by digital derivative spectrophotometry. The simultaneous determination of both drugs was performed by the zero-crossing method at 249.4 and 231.4 nm for dapsone and pyrimethamine, respectively. The best signal-to-noise ratio was obtained when the first derivative of the spectrum was used. The linear range of determination for the drugs was from 6.6×10^{-7} to 2.0×10^{-4} and from 2.5×10^{-6} to 2.0×10^{-4} mol/L for dapsone and pyrimethamine, respectively. The excipients of commercial pharmaceutical formulations did not interfere in the analysis. Chemical and spectral variables were optimized for determination of both analytes. A good level of repeatability, 0.6 and 1.7% for dapsone and pyrimethamine, respectively, was observed. The proposed method was applied for the simultaneous determination of both drugs in pharmaceutical formulations.

Malaria is endemic in Africa, southern and southeastern Asia, Central America, and northern South America. Chloroquinine is the selected drug used against *Plasmodium malariae*, *P. ovale*, *P. falciparum*, and *P. vivax*. Corticosteroids are contraindicated in the treatment of cerebral malaria. Infection with *P. falciparum* is resistant to chloroquinine and quinine sulfate oral treatments. If the illness is serious, intravenous injection with quinine or quinidine hydrochloride is mandatory. In case of risk, the treatment is usually supplemented with dapsone and pyrimethamine.

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Drugs such as dapsone (4,4′-sulfonylbisbenzamine) and pyrimethamine (5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidine diamine) (Figure 1) act synergistically in blocking enzymes responsible for folate metabolism in *P. falciparum*, the parasitic protozoan that causes the more severe form of malaria. Dapsone is also used in the treatment of leprosy.

Analytical determinations of dapsone, together with other drugs, are reported in biological fluids by liquid chromatography (LC; 1–3) and colorimetry (4). Dapsone has also been determined in tablets by nuclear magnetic resonance protonic (NMRH; 5). Pyrimethamine has been determined in pharmaceutical formulations by fluorometric methods (6). Dapsone and pyrimethamine have been determined individually in pharmaceutical formulations by nonaqueous thermometric titrimetry (7), and several methods have been reported for their simultaneous determination by LC in blood, plasma (8–12), and serum (13).

Taking into account the lack of official methods in the U. S. Pharmacopeia (14) for the simultaneous determination of dapsone and pyrimethamine, we report the development of a method of analysis by first-derivative spectrophotometry. This proposed method presents advantages over those found in the literature because it is direct, simple, inexpensive, rapid, and does not require sophisticated instruments. The sensitivity and selectivity are appropriate for the simultaneous determination of dapsone and pyrimethamine in pharmaceutical formulations. The use of derivative spectrophotometry permits resolution of the overlapped band and selection of the analytical wavelength to obtain good simultaneous determination of these drugs.

This technique has been used directly for simultaneous determination of organic (15, 16) and inorganic compounds (17–19) in many types of matrixes. The selection of the solvent and optimization of spectral variables are described to ensure precise procedures and accurate results in the application of the proposed method. The proposed method was successfully applied in simulated and commercial pharmaceutical formulations.

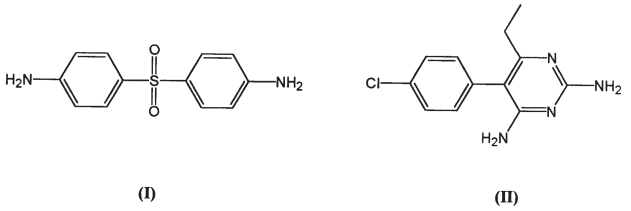

Figure 1. Structures of dapsone and pyrimethamine. (I) Dapsone (4,4′n-sulfonylbisbenzamine); (II) pyrimethamine [(5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidine diamine)].

Experimental

Instrumentation

A Shimadzu (www.shimadzu.com) UV-1603 spectrophotometer with 10 mm quartz cells was used for measurement of the absorbance and derivative absorption spectra. For all the tested solutions, first-derivative spectra were recorded over the range of 400–200 nm against acetonitrile. The spectral data were processed by the software Shimadzu kit version 3.7 (P/N 206-60570-04).

Materials and Reagents

All reagents were analytical grade. Stock solutions 1.0×10^{-3} mol/L dapsone (Aldrich Chemical Co., Milwaukee, WI) and pyrimethamine (Sigma Chemical Co., St. Louis, MO) were prepared by dissolving 6.22 ± 0.01 and 6.21 ± 0.01 mg of each compound in a 25 mL volumetric flask with acetonitrile as solvent. Other concentrations were prepared by appropriate dilution with the same solvent. To study the effect of solvent on the spectral behavior of the drugs, stock solutions containing 1.0×10^{-3} mol/L dapsone and pyrimethamine were prepared by dissolving the same amount of each drug in different solvents, such as ethanol, methanol, acetonitrile, dimethylsulfoxide (DMSO), dimethylformamide (DMF), and benzonitrile. Other ranges of concentrations were prepared by appropriate dilution with the respective solvent and suitable containers to minimize solvent evaporation. The commercial pharmaceutical formulations were made in Brazil: Far-Manguinhos-DAPSONE® donated by the 9ª Regional de Saúde, Fóz do Iguaçú, and Daraprim® of Zest Farmaceutica Ltda.

Determination of Dapsone and Pyrimethamine in Mixtures

Aliquots of stock solutions of dapsone and pyrimethamine were simultaneously diluted in acetonitrile to obtain a concentration of 2.0×10^{-5}–20.0×10^{-5} mol/L. The calibration curves were determined for each compound in presence of 6.0×10^{-5} mol/L of the other. In all cases the corresponding absolute values of the first-derivative spectra at 249.4 and 231.4 nm for dapsone and pyrimethamine, respectively, were obtained, and the values were plotted against the corresponding concentration.

Determination of Dapsone and Pyrimethamine in a Simulated Pharmaceutical Formulation

A simulated pharmaceutical formulation was prepared, containing 100.0 mg dapsone, 12.5 mg pyrimethamine, and 77.5 mg of a mixture of common tablet excipients (magnesium stearate 5%, lactose, starch, talc, and sodium dioctylsulfosuccinate 95%). A fraction of powder of 4–5 ± 0.01 mg of simulated formulation containing both drugs was accurately weighed and transferred to a 100 mL volumetric flask and dissolved in acetonitrile. The content of the flasks was shaken for 10 min. The suspension was later centrifuged and the supernatant solution was evaluated by first-order digital derivative spectrophotometry.

Simultaneous Determination of Dapsone and Pyrimethamine in Simulated Commercial Pharmaceutical Formulation

Twenty tablets of Far-Manguinhos-DAPSONE containing dapsone and 10 tablets of Daraprim containing pyrimethamine were weighed and powered. A powder fraction of 151.43 ± 0.01 mg Far-Manguinhos-DAPSONE and 62.59 ± 0.01 mg Daraprim were mixed and homogenized. An amount of powder weighing 6– 8 ± 0.01 mg was transferred to

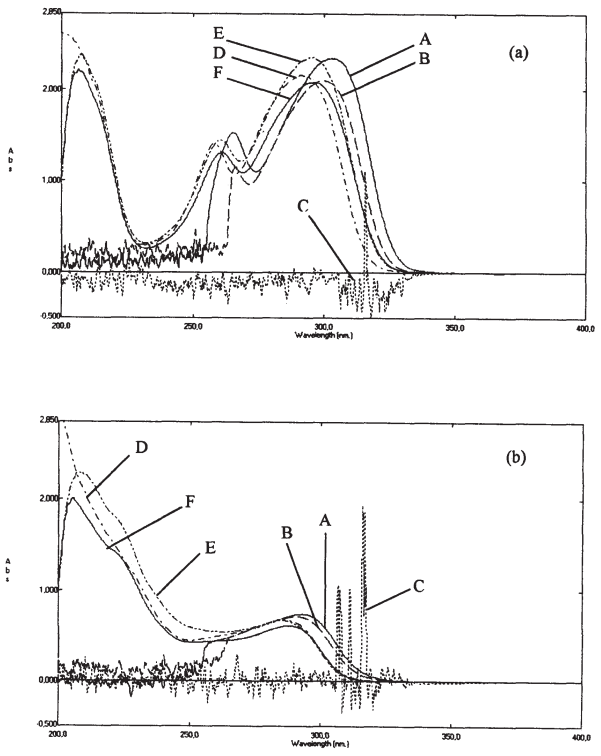

Figure 2. Spectra of dapsone and pyrimethamine in different solvents. (a) Dapsone, 8×10^{-5} mol/L and (b) pyrimethamine, 8×10^{-5} mol/L. (A) DMSO; (B) DMF; (C) benzonitrile; (D) acetonitrile; (E) methanol, and (F) ethanol.

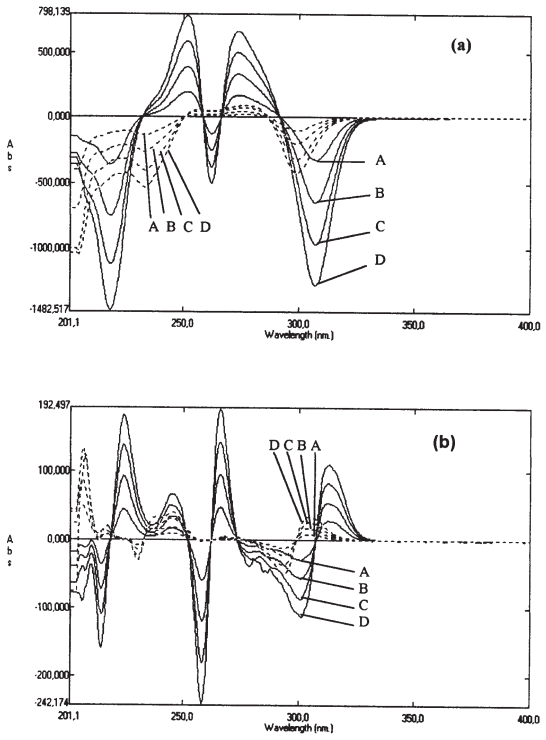

Figure 3. Derivative spectra of dapsone and pyrimethamine in different concentrations. (a) First derivative and (b) second derivative. Dapsone (—) and pyrimethamine (- - -). (A) 2×10^{-5} mol/L; (B) 4×10^{-5} mol/L; (C) 6×10^{-5} mol/L; (D) 8×10^{-5} mol/L.

a 100 mL volumetric flask and dissolved in acetonitrile. The contents of the flasks were shaken for 10 min. After the suspensions were centrifuged, the supernatant solution was evaluated by first-order digital derivative spectrophotometry.

Results and Discussion

Solvent Effect on Spectral Behavior of Dapsone and Pyrimethamine

The spectral behavior of 8×10^{-5} mol/L dapsone and 8×10^{-5} mol/L pyrimethamine was studied in different solvents (Figure 2). In DMSO the classic spectrum of dapsone shows 2 bands between 260 and 360 nm, and pyrimethamine shows 1 band centered in 290 nm. In this solvent, between the region of 200–250 nm, the signal-to-noise (S/N) ratio of both drugs is high. A similar spectral behavior of these compounds occurred in DMF (Figure 2B) because these solvents present a strong absorption band in this zone. With benzonitrile (Figure 2C) both compounds were completely decomposed, and the analytical signals were not observed; therefore, these solvents were discarded.

As shown in Figure 2, in acetonitrile, methanol, and ethanol, dapsone and pyrimethamine presented 3 and 2 bands, respectively. In acetonitrile, the bands are more sensitive and better defined. However, the bands of both compounds are totally overlapped between 200 to 320 nm. All these solvents could be used for the simultaneous determination if derivative spectrophotometry is used.

In 0.01M NaOH and 0.1M HCl, the bands corresponding to dapsone and pyrimethamine are very similar to those obtained in methanol. However, in 0.1M HCl, after 12 h the bands began to be altered: The first band of pyrimethamine decreased its height and the second band disappeared almost completely after 24 h, which could be attributed to decomposition of this drug. Dapsone spectrum was not altered in this medium. In neutral pH both drugs are insoluble. Taking these results into account, acetonitrile was selected as solvent.

Spectral Behavior

Evaluated directly against solvent, dapsone dissolved in acetonitrile showed 3 maximum absorption peaks at 200, 258, and 293 nm. Under similar conditions the pyrimethamine spectrum presented 2 absorption bands at 200 and 286 nm.

The spectral bands of both compounds are strongly overlapped. We adopted the digital derivative spectrophotometric mode proposed by Savitzky and Golay (20) because, with this mode, it was possible to resolve the spectral bands and obtain a noise control of the baseline, and, thus, good analytical signal.

Selection of Derivative Order

Different derivative orders of the spectra were obtained digitally from the zero-order spectra. Figure 3 shows that the first and second derivatives could be used for simultaneous determination of dapsone and pyrimethamine, because in all cases the derivatives present characteristic zones for each compound, which can be used for analytical purposes. When the derivative order increases, the sensitivity decreases. However, resolution of the spectra improves, thereby increasing the number of points in which each drug can be determined without mutual interference. Because the resolution was satisfactory, the first derivative was selected in favor of higher sensitivity.

Selection of the Smoothing and Scale Factors

For each compound, the first derivative was obtained by using a $\Delta\lambda$ value of 200 nm; the smoothing factor was varied; and the following values were used: 2, 4, 8, and 16. These val-

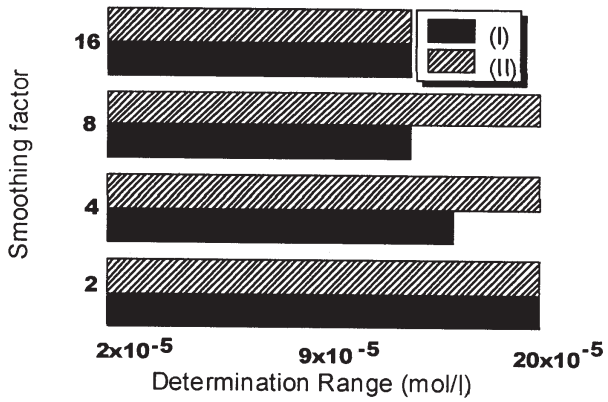

Figure 4. Effect of smoothing factor on determination range. Dapsone (I) and pyrimethamine (II).

Table 1. Validation parameters obtained with the proposed digital derivative spectrophotometric method[a]

Parameter	Dapsone	Pyrimethamine
Detection limit,[b] mol/L	2.0×10^{-7}	7.4×10^{-7}
Quantitation limit,[c] mol/L	6.6×10^{-7}	2.5×10^{-6}
Determination range, mol/L	6.6×10^{-7}–2.0×10^{-4}	2.5×10^{-6}–2.0×10^{-4}
Repeatability (RSD, %)	0.6	1.7
Regression lines	$h_1 = 9.13 \times 10^6$ C + 46.79	$h_2 = 6.87 \times 10^6$ C + 26.98
Correlation coefficient	r = 0.999	r = 0.999

[a] Where h, in derivative units, and C correspond to analyte concentration in mol/L.
[b] Criterion 3σ.
[c] Criterion 10σ.

ues are related to the range of wavelength in which the classic spectra are scanned and are defined by default by the software.

In general, when the smoothing factor is increased, the S/N ratio increases. When this factor is increased, the linearity of the calibration graph is lost at lower concentrations. By taking into account the concentration level of the analytes in pharmaceutical tablets, a study of this variable was necessary. Calibration curves of one analyte were determined in the presence of the other at constant concentration. Figure 4 shows that the determination range was greater for both drugs when a smoothing factor of 2 was used. At this value the S/N ratio was sufficiently high to obtain accurate results. Consequently, a smoothing factor of 2 was selected.

The scale factor must be studied to determine whether the system presents a distortion effect. Further, the selection of this parameter improves the reading of the analytical signal. A scale factor of 10^4 was selected for good reading of the analytical signal and elimination of distortion effects.

Selection of Analytical Wavelength

The first-derivative spectrum of dapsone dissolved in acetonitrile, evaluated directly against solvent, presented 2 zero-crossings at 231.4 and 290.7 nm; pyrimethamine also presented 2 zero-crossings at 249.5 and 284.3 nm. These points could be used to determine dapsone and pyrimethamine, respectively, by the zero-crossing approach (Figure 3). The wavelengths 249.4 and 231.4 nm were selected because, for both analytes, the sensitivities and precision were higher.

Stability

Different standard mixtures containing dapsone and pyrimethamine were exposed to daylight between 0 and 24 h. The samples were analyzed every hour by using the proposed method. In all cases, photochemical degradation was not evidenced because the analytical signals were not altered. Similar results were obtained by thermal degradation, which was studied

Table 2. Determination of dapsone and pyrimethamine in different standard mixtures

	Stated concentration ($\times 10^{-5}$M)		Found concentration[a] ($\times 10^{-5}$M; recovery, %)	
Ratio dapsone:pyrimethamine	Dapsone	Pyrimethamine	Dapsone	Pyrimethamine
1:1	6.00	6.00	5.96 ± 0.15 (99.30)	6.07 ± 0.10 (101.10)
1:2	4.00	8.00	3.98 ± 0.12 (99.50)	7.89 ± 0.13 (98.60)
1:3	2.00	6.00	2.03 ± 0.14 (101.50)	5.98 ± 0.15 (99.70)
1:4	2.00	8.00	1.98 ± 0.15 (99.00)	7.85 ± 0.12 (98.10)
1:5	2.00	10.00	1.95 ± 0.13 (97.50)	9.85 ± 0.12 (98.50)
1:8	1.00	8.00	0.99 ± 0.13 (99.00)	7.87 ± 0.10 (98.30)
2:1	2.00	1.00	1.96 ± 0.15 (98.00)	0.979 ± 0.10 (97.90)
4:1	4.00	1.00	3.98 ± 0.12 (99.50)	0.978 ± 0.13 (97.80)
6:1	6.00	1.00	5.89 ± 0.14 (98.16)	0.978 ± 0.15 (97.80)
8:1	8.00	1.00	7.84 ± 0.15 (98.00)	0.978 ± 0.12 (97.80)

[a] Mean of 5 determinations.

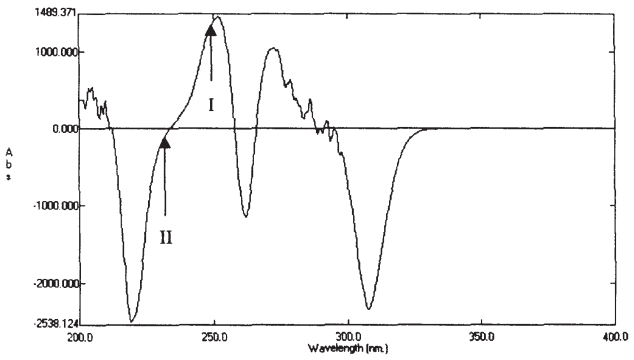

Figure 5. First-derivative spectrum of simulated Maloprim dissolved in acetonitrile. Determination of dapsone (I) and pyrimethamine (II).

by heating the solutions in a heating device for 1 h at 10–60°C. When the temperature was >60°C, the solvent evaporated.

When both drugs were subjected to acid and alkaline hydrolysis for 15 min at 50°C, only slight changes were observed, which can be attributed to the difference of absorption of acid and basic forms.

Analytical Features

Calibration curves of the first-derivative values versus the respective analyte concentrations were obtained for both analytes. All analytical features are shown in Table 1.

To establish the proportions on which the analyte can be measured accurately in the presence of the other analyte, recoveries were made of dapsone and pyrimethamine in different proportions in samples containing mixed standard solutions (Table 2). If the proportion of the dapsone:pyrimethamine concentrations was between 1:8 and 8:1, determination of the content of each compound was possible. The results showed that this method has a wide application range and that simultaneous determination of both drugs in pharmaceutical formulations is possible.

Application

The accuracy of the method was determined by analysis of simulated formulations samples according to the procedure described above. The recoveries were 101.2 ± 0.3 and 100.9 ± 0.3%, for dapsone and pyrimethamine, respectively. In this context, the excipients normally found in tablets did not interfere in the proposed method.

Because of government control in obtaining pharmaceutical formulations established in different countries, we could obtain only Brazil tablet formulations containing individual drugs.

The stability of simulated Maloprim was determined on Far-Manguinhos-DAPSONE and Daraprim, which contained 100 mg dapsone and 12.5 mg pyrimethamine, respectively. In simulated Maloprim, the effect of photochemical and thermal degradations was performed under conditions similar to those used for the standard solution. In all cases, the results showed that the analytical signals were clearly identical to those obtained with the standard solutions, which indicates that the excipients and degradation products did not affect the simultaneous determination. In this context, the results demonstrated the stability of the proposed method.

The first-derivative spectra of simulated Maloprim dissolved in acetonitrile is shown in Figure 5. It should be emphasized that the proposed method is possible only when the derivative mode is used. The amounts of analytes found in simulated Maloprim were 100.6 ± 0.7 and 12.3 ± 0.4 mg dapsone and pyrimethamine, respectively.

Acknowledgments

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