Determination of Attapulgite and Nifuroxazide in Pharmaceutical Formulations by Sequential Digital Derivative Spectrophotometry

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A new method for the sequential determination of attapulgite and nifuroxazide in pharmaceutical formulations by first- and second-derivative spectrophotometry, respectively, has been developed. In order to obtain the optimal conditions for nifuroxazide stability, studies of solvent, light, and temperature effects were performed. The results show that a previous hydrolysis of 2 h in 1.0×10^{-1}M NaOH solution is necessary in order to obtain stable compounds for analytical purposes. Subsequently, the first- and second-derivative spectra were evaluated directly in the same samples. The sequential determination of the drugs can be performed using the zero-crossing method; the attapulgite determination was carried out using the first derivative at 278.0 nm and the nifuroxazide determination, using the second derivative at 282.0 nm. The determination ranges were 5.7×10^{-6}–1.0×10^{-4} and 3.7×10^{-8} –1.2×10^{-4}M for attapulgite and nifuroxazide, respectively. Repeatability (relative standard deviation) values of 1.2 and 3.0% were observed for attapulgite and nifuroxazide, respectively. The ingredients commonly found in commercial pharmaceutical formulations do not interfere. The proposed method was applied to the determination of these drugs in tablets. Further, infrared spectroscopy and cyclic voltammetry studies were carried out in order to obtain knowledge of the decomposition products of nifuroxazide.

Nifuroxazide is a chemotherapeutic agent, derived from nitrofuran, which is extensively used in the treatment of diarrhea of bacterial origin because it is effective against most of the germs that habitually are present in intestinal infections. On the other hand, activated attapulgite corresponds to a magnesium and aluminum silicate of sedimentary or metamorphic origin, subjected to a high-temperature process (1). This salt acts as an adsorbent of exogenous or endogenous toxins that are products of pathogenic bacteria in the intestinal tract and are responsible for diarrhea and other gastrointestinal symptoms (2). Because of the antibacterial action of the nifuroxazide and the adsorption capacity of attapulgite, the pharmaceutical industry has produced medications that contain both active species for treatment of diarrhea.

Nifuroxazide determination in pharmaceutical formulation has been reported using polarography (3–5) and spectrophotometry (3). In biological samples, this drug has been determined by liquid chromatography (LC; 6, 7), adsorptive stripping voltammetry (8, 9), and electrochemistry with Sephadex-modifield carbon paste electrodes (10). On the other hand, the attapulgite identification in pharmaceutical products has been reported by X-ray diffraction (11). The U.S. Pharmacopoeia (12) does not report either the individual determination of nifuroxazide or any method for its simultaneous or sequential determination with attapulgite.

In this work, an accurate, precise, and inexpensive derivative spectrophotometry method is proposed for the sequential determination of both compounds in which hydrolysis in 1.0×10^{-1}M NaOH solution is required. After the hydrolysis process, the compounds are evaluated directly in the same sample by first- and second-order derivative spectrophotometry. A study of solvent, light, and temperature effects on the decomposition of nifuroxazide was performed, and optimization of the spectral variables and application of the proposed method for the determination of these drugs in tablets are also included in this work.

Experimental

Instruments

A Shimadzu UV-1603 spectrophotometer with 10 mm quartz cells was used for measurement of the absorbance and derivative absorption spectra. For all the tested solutions, the first- and second-derivative spectra were recorded over the 650.0–190.0 nm range against solvent. The spectral data were processed by Shimadzu kit Version 3.7 (P/N 206-60570-04) software, and a smoothing factor of 40 and a scale factor of 1.0×10^4 were used.

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Infrared spectra of samples were obtained with an FT-IR spectrometer Bruker Model Vector 22. Spectra in the range 4000 to 400 cm^{-1} were obtained with samples dispersed in KBr with a resolution of 4 cm^{-1}.

Cyclic voltammetric experiments were performed using a Voltammetric Analyzer CV–50W (Bioanalytical Systems, Lafayette, IN). A 3-electrode assembly was used for all measurements. Glassy carbon was employed as the working electrode, a saturated calomel electrode (SCE) as the reference, and a platinum coil as the counter electrode.

Reagents

All reagents were analytical reagent grade. Laboratorio Chile, Santiago, Chile, kindly provided attapulgite (I) and nifuroxazide (II).

Stock solutions of attapulgite and nifuroxazide were prepared by dissolving 46.1 ± 0.01 and 27.5 ± 0.01 mg, respectively, in 100 mL NaOH in order to give 1.0×10^{-3}M solution. Other concentrations were prepared by appropriate dilutions using the same solvent. The tablets containing (I) and (II) were also dissolved in the same solvent and then hydrolyzed.

Procedure for Hydrolysis of (I) and (II)

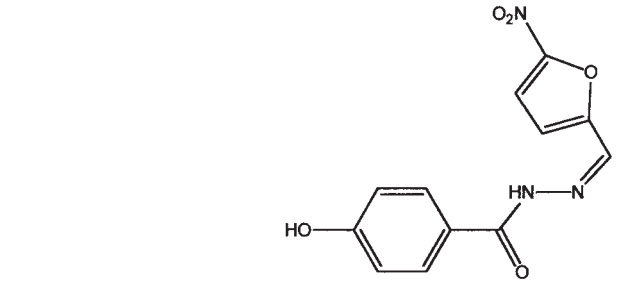
In a 400 mL beaker, 100 mL 1.0×10^{-4}M nifuroxazide and/or attapulgite dissolved in 1.0×10^{-1}M NaOH solution was hydrolyzed by heating and stirring at 90°C for 2 h. Then the solution was cooled and transferred to a 100 mL volumetric flask and diluted to the mark with 1.0×10^{-1}M NaOH solution.

Calibration Procedure for Determination of (I) and (II) in Mixtures

Aliquots of the stock solutions of attapulgite and nifuroxazide were simultaneously diluted in 1.0×10^{-1}M NaOH solution to obtain a concentration range of 2.0×10^{-5}–16.0×10^{-5}M. The calibration graphs were determined for each compound in presence of 1.0×10^{-5}M of the other. In all cases, the solutions were heated up 90°C for 2 h, cooled, and transferred to a 100 mL volumetric flask, and diluted to the mark with 1.0×10^{-1}M NaOH solution. The corresponding absolute values of the first-derivative spectra at 278.0 nm, and the second-derivative spectra at 282.0 nm for attapulgite and nifuroxazide, respectively, were obtained, and the values were plotted against the corresponding concentrations.

Procedure for Determination of (I) and (II) in Tablets

Twenty tablets of each formulation were weighed and powdered. A quantity of powder equivalent to 9–12 mg tablet containing (I) and (II) were accurately weighed and then heated for 2 h in 1.0×10^{-1}M NaOH solution as indicated above. The contents of the flasks were shaken and stirred for 20 min, and then the first- and second-derivative spectra were obtained.



$(Mg,Al)_2Si_4O_{10}(OH)_4H_2O$

(I) (II)

Figure 1. Structures of attapulgite (I) and nifuroxazide (II).

Results and Discussion

Attapulgite and nifuroxazide (Figure 1) are pharmaceutically active compounds of inorganic and organic nature, respectively. For this reason, the structures of these compounds are quite different. In this context, the spectral behavior and the solubility of both compounds can be expected to be quite different. According to its structure, the solubility of nifuroxazide in organic solvent is large, but attapulgite is not soluble in this type of solvent. For this reason, it was necessary to assess other alternative solvents such as acids and alkalis.

As a result of the study of solvent effects on the spectral behavior in the development of this method, 1.0×10^{-1}M NaOH solution was selected as the solvent for dissolving these drugs. Under these conditions, the best solubility of both drugs is achieved and the spectra are better defined (Figure 2). This solvent is also economical and common in analytical laboratories.

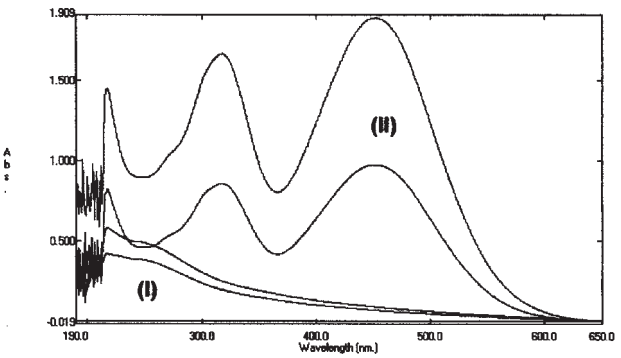


Figure 2. Zero-order spectra of attapulgite and nifuroxazide in 1.0×10^{-1}M NaOH solution. (I) Attapulgite: 8.0×10^{-5} and 1.0×10^{-4}M; (II) nifuroxazide: 5.0×10^{-5} and 1.0×10^{-4}M.

In the spectral study, it was observed that the spectra of these drugs are totally overlapped. Accordingly, it is necessary to use derivative spectrophotometry techniques in order to resolve the spectra (13, 14).

Stability of the Drugs

It was observed that nifuroxazide had a yellow color in acid medium. On the other hand, in basic medium it changed to a reddish brown color, which was intensified when the concentration of the drug was increased. Under these conditions, it was supposed that the drug was severely decomposed in a short period of time. In order to explain this decomposition, a study of the stability of the drug related to temperature and light influences was carried out.

Photostability of nifuroxazide.—Because this compound is photosensitive (12, 15), the effect of the direct sunlight on a 1.0×10^{-4}M solution of nifuroxazide was studied in basic medium at room temperature. In few hours the color of the solution changed from reddish brown to clear yellow. On the other hand, the band located between 500.0 and 400.0 nm decreased and a discrete increase of the maximum located between the 370.0 and 300.0 nm was observed (Figure 3), which would indicate a structural change of the compound. Probably the molecule underwent a hydrolysis reaction that modified considerably the electronic density associated with the nitro group. An identical behavior was observed when the drug was exposed to indirect laboratory light.

Stability of nifuroxazide with the temperature.—Taking into account that nifuroxazide is unstable, a study of the temperature effect on the stability of this drug was also carried out. Different solutions were heated at 90°C, and spectra were recorded each 5 min. According to Figure 4, it can be observed that after 60 min nifuroxazide decomposition was almost complete; however, in order to ensure that the decomposition is quantitative, 2 h of heating to 90°C was selected.

The same study was carried out heating the solution for 2 h at temperatures between 40°–80°C. The results show that in all cases nifuroxazide was only partially decomposed and that 90°C is required to reach full decomposition of nifuroxazide.

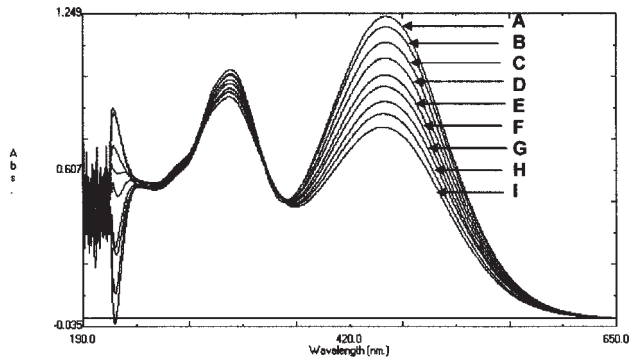


Figure 3. Spectra of nifuroxazide for different times of exposure to direct sunlight: (A) 0 h, (B) 1 h, (C) 2 h, (D) 3 h, (E) 4 h, (F) 5 h, (G) 6 h, (H) 7 h, (I) 8 h.

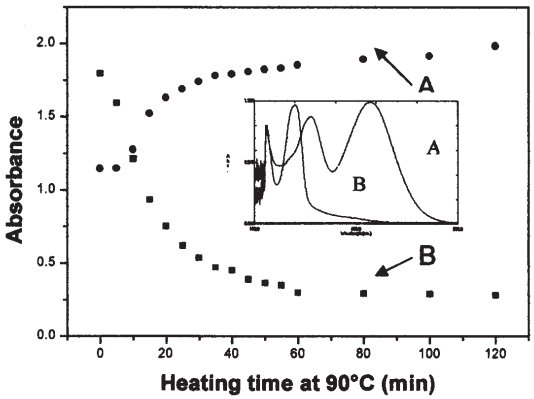


Figure 4. Effect of the heating time at 90°C on the nifuroxazide spectrum: (A) absorbance at 278.0 nm and (B) absorbance at 448.5 nm. Inset: Zero-order spectra of nifuroxazide, 1.0×10^{-4}M. (A) Recently prepared and (B) after 2 h of heating.

On the other hand, this drug was stable at 4°C because it was observed that after 8 h the spectra were unaltered and the reddish brown color of the solution did not change. However, this experimental condition is not appropriate for analytical purposes.

In summary, temperature is a decisive factor in the decomposition of the drug. When the drug is heated at 90°C for 2 h, it is decomposed completely, giving rise to stable products as can be seen in Figure 4.

Decomposition of nifuroxazide.—When nifuroxazide was subjected to alkaline hydrolysis with 1.0×10^{-1}M NaOH solution for 2 h, its UV-visible spectrum was strongly altered. Two bands were present in the UV region at 238.0 and 280.0 nm, and the band at 450.0 nm, attributable to the nitro group of the initial nifuroxazide, totally disappeared (Figure 4). This drastic spectral change evidenced a structural transformation as a result of the hydrolysis in alkaline medium.

These structural changes are also evidenced in the infrared (IR) spectra (Figures 5 and 6) by the disappearance of the band corresponding to the symmetrical (ν_s 1363 cm^{-1}) and asymmetric (ν_s 1509 cm^{-1}) vibrations of the nitro group. On the other hand, the band assigned to the deformation of the furan ring (1266 cm^{-1}) also disappears; however, an aromatic character still persists in the spectrum of the hydrolyzed product, with bands at 1445 cm^{-1} assigned to a ν C-C aromatic carbon and at 862 cm^{-1} to a δ CH mode, both corresponding to a phenyl fragment.

With the purpose of confirming the disappearance of the nitro group, cyclic voltammograms of recently prepared nifuroxazide and hydrolyzed nifuroxazide were recorded using a supporting electrolyte of 1.0×10^{-1}M tetraethyl ammonium chloride solution–pyridine–formic acid volume ratio (12 + 2 + 1, v/v). This electrolyte was used earlier for this type of compound (16). It was found that nifuroxazide presents a peak at –0.45 V vs SCE, which is typical of the reduction of a nitro group and is evidence that this functional

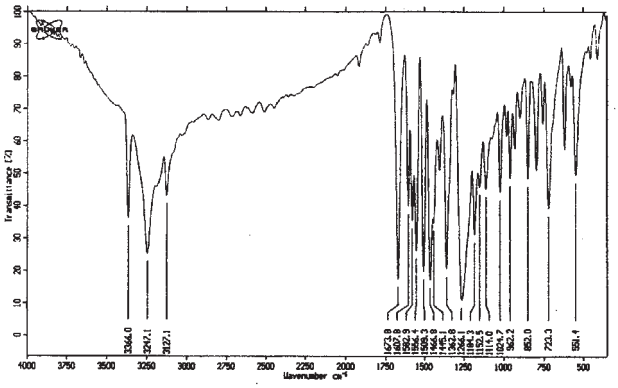


Figure 5. IR spectrum of nifuroxazide recently prepared.

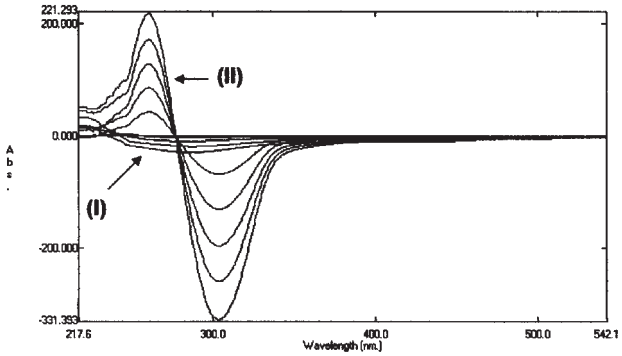


Figure 7. First-derivative spectra of attapulgite (I) and nifuroxazide (II) after alkaline hydrolysis. (I) 2.0, 4.0, 6.0, 8.0×10^{-5}M; (II) 2.0, 4.0, 6.0, 8.0, 10.0×10^{-5}M.

group is present in the original compound. On the other hand, the cyclic voltammograms of the compound previously hydrolyzed in the same supporting electrolyte do not contain the typical signal for the reduction of a nitro group; this indicates that the product of decomposition of the resulting hydrolysis compound does not contain a nitro group.

A study of the spectral behavior in the UV region of the hydrolyzed compound showed that the spectrum is well defined and identical to that observed for phenol. The spectra remain stable and reproducible for different hydrolyzed samples. Therefore, it is possible to use this spectrum for analytical purposes.

Stability of attapulgite.—The same studies were carried out for attapulgite. The results showed that this compound remained stable under all conditions; it did not change visually, and the spectral behavior was not altered.

Selection of Spectral Parameters

Order of the derivative and analytical wavelength.—To select the order of the derivative in the simultaneous determinations of each drug, the first up to the fourth derivatives were registered. When the first derivative was used, nifuroxazide showed a zero crossing at a wavelength of 278.0 nm (Figure 7). However, the attapulgite did not show a zero crossing. For this reason, the first derivative was discarded for simultaneous determination of both compounds.

In the second-derivative graph, the nifuroxazide showed 2 zero crossings at 263.0 and 305.2 nm, and the attapulgite showed 1 zero crossing at 282.0 nm (Figure 8). Despite the fact that both drugs showed this feature in second derivative, it is not possible to determine both drugs simultaneously. The explanation is that the nifuroxazide zero crossings are located in an area where the attapulgite does not show a quantifiable band. The third and fourth derivatives were discounted because both present low signal-to-noise (S/N) ratios. It was decided to carry out sequential determination of attapulgite and nifuroxazide by means of the first and second derivative at 278.0 and 282.0 nm, respectively.

Smoothing and amplification factors.—The smoothing factors are defined by default by the software that carries out the derivation, according to the range of wavelengths used for the classic spectrum sweeping. In this work, different values for smoothing factors were used among those given by the equipment (5, 10, 20, 40); the last one was selected because it

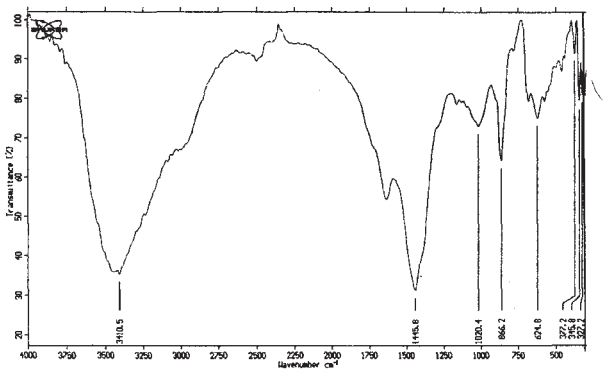


Figure 6. IR spectrum of nifuroxazide after 2 h of heating.

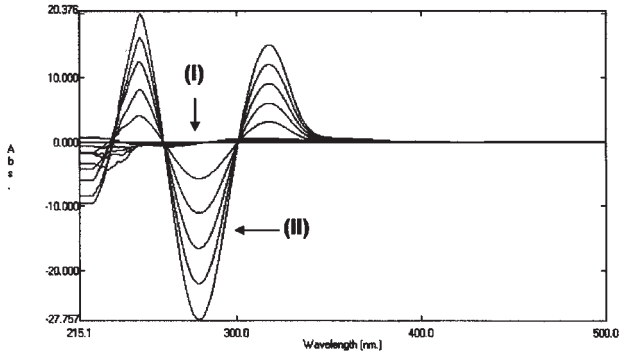


Figure 8. Second-derivative spectra of attapulgite (I) and nifuroxazide (II) after alkaline hydrolysis. (I) 2.0, 4.0, 6.0, and 8.0×10^{-5}M; (II) 2.0, 4.0, 6.0, 8.0, and 10.0×10^{-5}M.

Table 1. Analytical features

Analytical parameters	Attapulgite	Nifuroxazide
Detection limit[a], M	$1.7 \times 1\ 0^{-6}$	1.1×10^{-8}
Determination limit[b], M	5.7×10^{-6}	3.7×10^{-8}
Range of determination, M	5.7×10^{-6}–1.0×10^{-4}	3.7×10^{-8} –1.2×10^{-4}
Repeatability (RSD[c], %)	1.2	3.0

[a] 3σ criterion.

[b] 10σ criterion.

[c] RSD = Relative standard deviation.

gave the best S/N ratio, and the spectra had good resolution. An amplification factor of 10^4 was selected to enlarge the signal of attapulgite in order to facilitate the measurement and to diminish error in reading the signal.

Analytical Features

Calibration graphs of each drug in presence of the other were produced. By using the first derivative, a smoothing factor of 40, a scale factor of 1.0×10^4, and $\Delta\lambda$ of 460.0 nm, the calibration graph for attapulgite was obtained by plotting the first-derivative value, h_1 (λ = 278.0 nm), versus the respective analyte concentration. For nifuroxazide, the calibration graph was obtained using the same $\Delta\lambda$, smoothing, and scale factor, but the second-derivative value, h_2, at λ = 282.0 nm, was plotted versus nifuroxazide concentration. The linear regression equations and the correlation coefficients calculated for mixtures of both analytes are the following:

$$h = 50\ 500 \times C\ (mol/L) + 0.590$$
$$r = 0.9997$$
$$\lambda = 278.0\ nm$$

$$h = 26\ 7931 \times C\ (mol/L) + 0.172$$
$$r = 0.9999$$
$$\lambda = 282.0\ nm$$

where h is in derivative unit (DU) and C correspond to the analyte concentration in M. A good linearity for both compounds was obtained, and the analytical signals did not present mutual interference for the determination of these drugs. All analytical features are shown in Table 1.

In order to establish the ratios at which one analyte can be accurately measured in presence of the other, the recoveries of samples containing standard solutions of mixtures of attapulgite and nifuroxazide in different concentration ratios were carried out. The results are shown in Table 2. The content of each compound can be determined if the concentration ratio is between 1:8 to 8:1 for attapulgite:nifuroxazide. According to the results, it is possible to conclude that this method has a wide range of application and permits the simultaneous determination of both compounds in real pharmaceutical formulations, which contain a 1:1.75 ratio.

Application

The accuracy of the proposed method using a previous hydrolysis was determined by analysis of synthetic formulation samples containing 200 mg nifuroxazide and 350 mg attapulgite mixed with 163 mg excipients (magnesium stearate + gelatin 3–5% and lactose–starch 95–97%). The recoveries were found to be 98.9 ± 0.9 and 99.3 ± 0.6% for attapulgite and nifuroxazide, respectively. These results show that common excipients found in tablets do not interfere in the proposed method.

In addition, the content of attapulgite and nifuroxazide in Diarfin (Laboratorio Chile S.A.) and Diaren (Laboratorio Saval Eurolab) were determined. The pharmaceutical formulations contain nominally 200 mg nifuroxazide and 350 mg attapulgite. Following application of the proposed method, average results for nifuroxazide in Diarfin and Diaren were 195 ± 0.01 and 197± 0.01 mg, respectively, and for attapulgite 348 ± 0.01 and 349 ± 0.01 mg, respectively.

Acknowledgments

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Table 2. Percentages of recovery from synthetic samples having different amounts of attapulgite and nifuroxazide

Nifuroxazide:attapulgite	Attapulgite added, g	Nifuroxazide added, g	Attapulgite found[a] recovery, %	Nifuroxazide found[a] recovery, %
1:8	2.31×10^{-4}	1.85×10^{-3}	$(2.31 \pm 0.02) \times 10^{-4}$ (100)	$(1.81 \pm 0.01) \times 10^{-3}$ (98.9)
1:6	2.31×10^{-4}	1.39×10^{-3}	$(2.28 \pm 0.02) \times 10^{-4}$ (98.7)	$(1.36 \pm 0.01) \times 10^{-3}$ (98.6)
1:4	2.31×10^{-4}	9.24×10^{-4}	$(2.28 \pm 0.01) \times 10^{-4}$ (98.7)	$(9.24 \pm 0.01) \times 10^{-4}$ (99.6)
1:2	2.31×10^{-4}	4.62×10^{-4}	$(2.30 \pm 0.02) \times 10^{-4}$ (99.6)	$(4.60 \pm 0.01) \times 10^{-4}$ (99.6)
1:1.75	4.04×10^{-4}	2.31×10^{-4}	$(4.0 \pm 0.01) \times 10^{-4}$ (99.0)	$(2.31 \pm 0.01) \times 10^{-4}$ (100)
1:1	2.75×10^{-4}	2.75×10^{-4}	$(2.74 \pm 0.01) \times 10^{-4}$ (99.6)	$(2.75 \pm 0.01) \times 10^{-4}$ (100)
6:1	1.70×10^{-3}	2.75×10^{-4}	$(1.70 \pm 0.02) \times 10^{-3}$ (100)	$(2.76 \pm 0.01) \times 10^{-4}$ (100.4)
8:1	2.20×10^{-3}	2.75×10^{-4}	$(2.20 \pm 0.02) \times 10^{-3}$ (100)	$(2.76 \pm 0.01) \times 10^{-4}$ (100.4)

[a] Average of 5 determinations.

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