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First-derivative spectrophotometric and LC determination of nifedipine in Brij® 96 based oil/water/oil multiple microemulsions on stability studies

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Abstract

A first-derivative spectrophotometric ($^1D_{387}$) method was developed for the determination of nifedipine in oil/water/oil (O/W/O) multiple microemulsions during stability studies. The UV first-derivative spectra were recorded over the wavelength range 200–600 nm ($\Delta\lambda = 16$). The derivative procedure was based on the linear relationship between nifedipine concentration and the first-derivative amplitude at 387 nm. This method was validated and compared with a liquid chromatography (LC) procedure used for the quantitative analysis of the drug. Both methods showed excellent precision and accuracy with values of 2.09 and 1.82%, respectively, for the LC method and of 1.53 and 1.64%, respectively, for the $^1D_{387}$ method. The established linearity range was 5–30 μg ml^{-1} with r^2 values of 0.9980 and 0.9988 for LC and first-derivative procedures, respectively. Nifedipine recoveries from spiked placebos were $>95\%$ for both methods over the linear range analysed. These methods have been successfully used for determining of nifedipine content of multiple microemulsions during stability studies, since there was no interference with its decomposition products.

Keywords: High performance liquid chromatography; Derivative UV spectrophotometry; O/W/O multiple microemulsion; Nifedipine; Validation; Stability studies.

1. Introduction

Nifedipine, [1,4-dihydro-2,6-dimethyl-4-(2-nitro-phenyl)-3,5-pyridine dicarboxylic acid dimethyl ester] (Fig. 1), is a Ca^{2+}-channel blocker that inhibits the transmembrane influx of Ca^{2+} into cardiac muscle cells and vascular smooth muscle through specific ion channels. It induces relaxation of smooth muscle and decreases peripheral vascular resistance [1,2] for which it is widely used for the treatment of hypertension, angina pectoris and other cardiovascular disorders [3,4]. After oral administration nifedipine is rapidly and almost completely absorbed but undergoes extensive first pass metabolism in man [5,6]. Nifedipine is highly sensitive to chemical oxidation and so, depending on the source of

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irradiation, two oxidation products of nifedipine have been reported. One is the nitrophenylpyridine elicited by ultraviolet light and the other, nitrosophenylpyridine product is caused by daylight irradiation (i.e. daylight) [7,8]. It has also been reported that light irradiation of ethanolic solutions of nifedipine convert this compound to the fully aromatic nitroso derivatives [9].

Many methods have been reported for the determination of nifedipine in biological fluids, mainly involving gas-chromatography (GC) [10,11], high performance liquid chromatography (LC) with either UV detection [12–15] or electrochemical detection[16,17] and fluorescence procedures [18]. However, some of these HPLC methods employ electrochemical detection for quantifying low concentrations of the drug in biological fluids, and so have the disadvantage of being expensive and not always available in many laboratories. On the other hand, GC has serious drawbacks due to thermal degradation of nifedipine during its analysis at high temperatures.

In the last years, some methods have been developed for the determination of nifedipine in pharmaceuticals [19–21]. Nevertheless, none of them have been described with the aim of quantifying nifedipine in the presence of its degradation products during stability studies. Due to this, a novel, rapid and specific UV spectrophotometric method is reported and compared with an UV detection RP-HPLC method described by Grundy et al. [22] for the determination of nifedipine within a novel controlled release dosage form of nifedipine, intended for transdermal administration, carried out by our group in order to avoid the high first pass effect suffered by the drug when it is administered orally [5,6]. This kind of systems could permit to reduce the daily dose taken by the patients and so facilitate their dosage compliance and could also incorporate drug in the external oil phase that might avoid the lag time that appears at the beginning of the in vitro permeation experiments that has been noticed with O/W microemulsions [23].

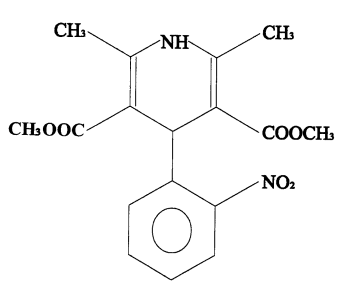

Fig. 1. Chemical structure of nifedipine.

This new first-derivative analytical procedure has been successfully applied for the quantitation of nifedipine during stability studies in contrast with other conventional spectrophotometric procedures that cannot be use in this kind of studies. The use of first-derivative spectroscopy allows as well a simple, quick and accurate determination of nifedipine without any preliminary separation procedures that might be required in some of the aforementioned methods, which are not always suitable for routine analysis in pharmaceutical industry as they are always time consuming and involve the use of expensive equipments not always available in many quality control laboratories.

2. Experimental

2.1. Chemicals

Nifedipine was purchased from Sigma Chemicals (Madrid, Spain). Microemulsion components were isopropyl myristate (Merck Chemicals, Barcelona, Spain), Brij® 96 V (Sigma Chemicals, Madrid, Spain), propylene glycol (PG) and polyethylene glycol 400 (PEG 400) (Panreac, Madrid, Spain). Other reagents used were HPLC grade methanol (Sigma Chemicals, Madrid, Spain), analytical grade acetic acid and triethylamine (TEA) (Panreac, Madrid, Spain). Deionised water was used for microemulsion formulation and HPLC mobile phase and was obtained from a Milli-Q Plus system (Millipore, Madrid, Spain).

2.2. Apparatus and conditions

2.2.1. First-derivative spectrophotometric system

Absorption and first-derivative spectra were recorded over the wavelength range 200–600 nm in 1 cm quartz cells using a Beckman DU®-7 spectrophotometer. The first-derivative spectra were obtained at a slit width ($\Delta\lambda$) of 16 nm. The scan speed was of 600 nm min^{-1}. Both spectra were carried out using methanol as a blank.

2.2.2. HPLC-UV system

The HPLC method used was based on the method developed by Grundy et al. [22] which has been employed to quantify in vitro release of nifedipine from gastrointestinal therapeutic systems. This method was also able to quantify the drug in the presence of the microemulsion excipients and drug decomposition products without any interference and so it was used to compare the results obtained with the first-derivative spectrophotometric ($^1D_{387}$) method.

For this HPLC technique, a Hewlett-Packard system consisting of a HP 1050 quaternary pump with a HP 1050 programmable multiple wavelength detector, set at 350 nm, were used. Chromatograms were recorded and the peak area responses were measured using a HP 3396 Series II Integrator. The separation was carried out at room temperature, on a reverse phase Tracer-Extrasil Column of 150 × 4.0 mm ID and 5 μm particle size (Teknokroma, Madrid, Spain). The mobile phase consisted of methanol-water (65:35, v/v) adjusted to approximately pH 4.0 with acetic acid and TEA as 1 and 0.03% final concentration, respectively. The mobile phase was filtered through 0.45 μm nylon filters, degassed and pumped at 0.9 ml min^{-1}. Sample preparation and analysis were conducted under sodium lamps. The injection volume was of 20 μl for all standards and samples.

2.3. Standard preparations

For the first-derivative and HPLC measurements, a stock solution was prepared by accurately weighing 50 mg of nifedipine into a 50 ml volumetric flask, dissolved and diluted to volume with methanol to obtain a concentration of 1 mg ml^{-1}.

Table 1
Composition (% w/w) of placebo and nifedipine O/W primary microemulsion and placebo and nifedipine O/W/O multiple microemulsion

	Placebo primary microemulsion	Nifedipine primary microemulsion
O/W primary microemulsion		
Drug	–	–
Nifedipine	–	1.8
Excipients		
Isopropyl myristate	15	15
Brij® 96 V	26.25	26.25
Propyleneglycol	8.75	8.75
Distilled water	48.2	48.2
	Placebo multiple microemulsion	Nifedipine multiple microemulsion
O/W/O multiple microemulsion		
Nifedipine Primary Microemulsion	–	20
Placebo Primary Microemulsion	20	–
Excipients		
Brij® 96 V	22.5	22.5
Polyetilenglycol 400	7.5	7.5
Isopropyl myristate	50	50

The stock solution was further diluted with methanol to reach a concentration range of 5–30 μg ml^{-1}.

2.4. Sample solutions

For both analytical methods, 10 ml of nifedipine multiple microemulsion (composition shown in Table 1) containing 36 mg of such drug, were stored at 50 °C for 5 days, 50 °C for 7 days and room temperature under daylight for 5 days to obtain 20, 35% and totally decomposed nifedipine, respectively. 5.55 ml of each nifedipine loaded multiple microemulsion (containing 20 mg of such drug), stored under these conditions, were transferred into a 100 ml volumetric flask and diluted to volume with methanol. Finally, 20, 35% and

totally decomposed nifedipine sample solutions were obtained by pipetting 1 ml of the earlier solutions into 10 ml volumetric flask and diluted to volume with methanol.

In order to prepare totally decomposed nifedipine in methanol, 50 mg of the drug were transferred into a 50 ml volumetric flask and diluted with methanol. This sample solution was stored at room temperature under daylight for 5 days. After that, further dilutions were made to reach a final theoretical nifedipine concentration of 20 μg ml^{-1}.

2.5. Calibration

Aliquots of the standard stock solution of nifedipine were pipetted into different 100 ml volumetric flasks and diluted to volume with methanol. The final concentrations of nifedipine were in the range 5–30 μg ml^{-1} for the $^1D_{387}$ and HPLC methods. Each solution was analysed in triplicate for both methods. Peak areas were recorded at 350 nm and absorbences were measured at 387 nm for each procedure, respectively.

2.6. System suitability test

For the HPLC method, the system suitability test was evaluated by making 10 replicate injections of the standard and recording the peak responses. The systems was deemed suitable for its use if the coefficient variation was $< 3\%$ and the tailing factor $< 1.5\%$ [24].

2.7. Procedure

For the HPLC method, six injections of the standard and three injections of sample preparations (each 20 μl) were chromatographied. For the $^1D_{387}$ method, six measures of the standard solutions and three of the sample preparations were carried out. The amount of nifedipine (mg ml^{-1}), was calculated for both methods by the following formula:

$$\text{Amount of nifedipine (mg ml}^{-1}\text{ multiple microemulsion)} = (R_{sam}/R_{std})C \times F \times 10^{-3}$$

where R_{sam} and R_{std} are the average peak responses and the absorbance of the sample preparation and standard preparation, respectively, C the concentration (μg ml^{-1}) of standard preparation and F is the dilution factor.

3. Results and discussion

Due to the considerable overlapping of the microemulsion excipients in the region 200–280 nm for UV conventional and first-derivative spectra, it was necessary to compare the spectra of nifedipine O/W/O multiple microemulsion sample solutions and nifedipine in methanol solutions with both techniques, in order to demonstrate that not only microemulsion excipients but also drug decomposition products interfered in certain regions of UV conventional and $^1D_{387}$ nifedipine spectra.

Fig. 2(A) shows the zero order spectrum of nifedipine (20 μg ml^{-1}) and totally decomposed nifedipine in methanol standard solutions within the 200–600 nm wavelength region. As can be seen, nifedipine (20 μg ml^{-1}) spectra showed two maxima at 237 and 350 nm. In this figure, the evolution of UV conventional spectra of nifedipine in methanol solutions under fixed stability conditions can be observed, in which the absorption band at 350 nm totally disappeared and a new band set at 290 nm appeared. As can thus be observed, decomposed nifedipine spectrum displayed overlapping in the 200–440 nm region, which made the determination of nifedipine in the presence of its decomposition products unable to be carried out without interference from these products.

Fig. 2(B) shows the zero order spectra of nifedipine (20 μg ml^{-1}), 20% decomposed nifedipine and totally decomposed nifedipine within O/W/O multiple microemulsions in the same wavelength region as Fig. 2(A). As can be seen, there was a considerable overlapping between the three spectra. Nifedipine sample solutions (100% nifedipine and 20% decomposed nifedipine) showed two maxima at the same wavelength as 20 μg ml^{-1} standard solution spectra in Fig. 2(A).

The enhancement of absorbance for the three spectra seen in Fig. 2(B) at 237 nm, in comparison with spectra observed in Fig. 2(A) was due to the overlapping of the formulation excipients in the 200–280 nm region. As a result of excipient overlapping, in Fig. 2(B) the interference of nifedipine decomposition products could not be noticed over the wavelength range 200–280 nm as could be seen in Fig. 2(A).

The overlapping displayed by the zero order absorption spectra of 20% decomposed nifedipine Fig. 2(B) in the wavelength range was assessed due to the absorptivity of the decomposition products at the two working wavelengths ($\lambda = 237$ and 350 nm). Since the absorbance values at the two maxima for the zero order spectra of nifedipine (20 μg ml^{-1}) and 20% decomposed nifedipine sample solutions were very similar, it, therefore, was impossible to determine the real concentration of nifedipine in the presence of its decomposition products by measuring its absorbance without interference within the 200–440 nm range in the original zero order spectra.

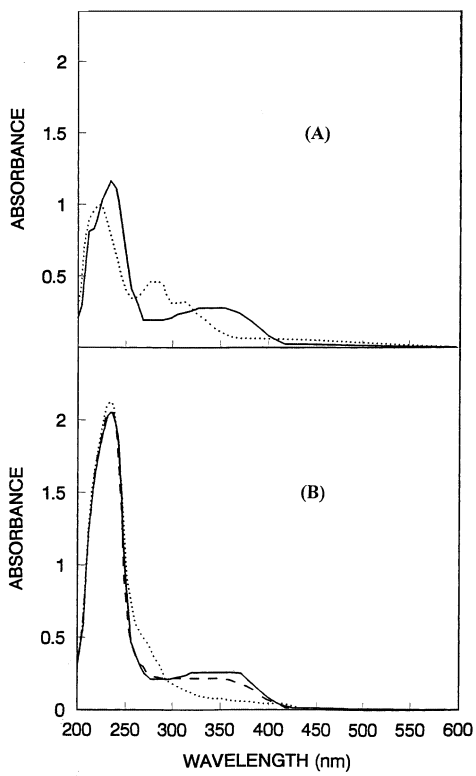

Fig. 2. (A) Zero order spectra of nifedipine (—), and totally decomposed nifedipine (• • •) from 20 g ml^{-1} methanol solutions; (B) Zero order spectra of nifedipine (—), 20% decomposed nifedipine (- - - -) and totally decomposed nifedipine (• • •) loaded O/W/O multiple microemulsions (3.6 mg ml^{-1}) in methanol (20 μg ml^{-1}).

For the conditions used in this work, the second derivative spectra of nifedipine (20 μg ml^{-1}) and 20% decomposed nifedipine sample solutions were poorly resolved. The only maximum on the second derivative spectra in which it was possible to determine nifedipine, was at 249 nm. However, at this wavelength interferences with drug decomposition products could be observed as well as a poorly linear relationship between spectrophotometric responses and nifedipine concentrations (data not shown).

In contrast, $^{1}D_{387}$ method offered an extremely valuable mean for the determination of nifedipine in presence of its decomposition products in multiple microemulsions, since this procedure allowed the quantitation of the drug at 387 nm. As it can be seen in Fig. 3(A), totally decomposed nifedipine samples did not show any interference at the selected wavelength ($\lambda = 387$ nm). Fig. 3(B) shows that 20% decomposed nifedipine could be clearly quantified at 387 nm, where first-derivative absorbance values for partially decomposed nifedipine showed a significant decrease in relation to first-derivative absorbance values for nifedipine (20 μg/ml). As can also be observed comparing the spectra show in Fig. 3(A, B), there was considerable overlapping between the microemulsion excipients and the drug in wavelength region of 200–280 nm and so it was impossible to use the wavelength of 246 nm for the determination of nifedipine in this kind of disperse systems. This excipient overlapping did not allow as well to observe the interference caused by the drug decomposition products over the 200–340 nm region as could be demonstrated in Fig. 3(A), in which there was no presence of the formulation excipients. Therefore, UV the first-derivative spectrophotometry could be used to quantify nifedipine in multiple microemulsion (in this work) or in bulk material (data not shown) during stability studies in which the drug is converted into decom-

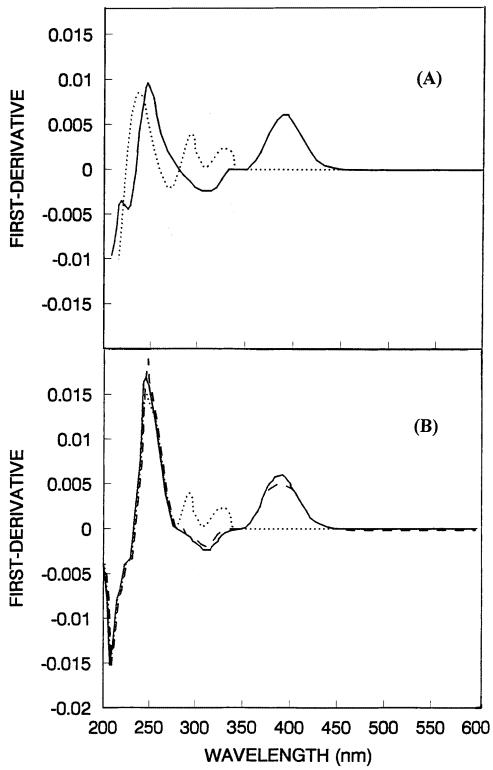

Fig. 3. (A) First-derivative spectra of nifedipine (—), and totally decomposed nifedipine (• • •) from 20 g ml^{-1} methanol solutions; (B) First-derivative spectra of nifedipine (—), 20% decomposed nifedipine (- - - -) and totally decomposed nifedipine (• • •) loaded O/W/O multiple microemulsions (3.6 mg ml^{-1}) in methanol (20 μg ml^{-1}).

position products. The wavelength of 387 nm was selected as the optimum working parameter in which the measurements taken gave the best linear response to the analyte concentration.

The HPLC method developed by Grundy et al. [22] and used in this work, did not allowed the detection of degradation products although it was a useful technique to quantify nifedipine during the stability studies carried out in this paper. UV detection at wavelengths equal or lower than 254 nm is commonly applied in existing nifedipine assays and is advantageous for the optimised detection of nifedipine metabolites and photodecomposition products [25,26]. However, significant baseline noise was noticeable in published chromatograms from several of these methods, which could compromise the accuracy of this method [25,26], so the wavelength of 350 nm was then used in this work. Fig. 4 shows HPLC chromatograms of nifedipine (20 μg ml^{-1}) (A) and 20% decomposed nifedipine (B) with retention times of 4.488 and 4.479, respectively. The degradation of nifedipine was noticed by a decrease in the peak area of the drug. Fig. 4(C) shows the chromatogram of totally decomposed nifedipine in which no peak of the drug was detected during the chromatogram running time (20 min).

3.1. Precision

The system precision was determined for both the HPLC and spectrophotometric methods by chromatographing six injections and measuring the spectrophotometric responses of the standard (20 μg ml^{-1}) within the same day (repeatability) and three determinations of three different standard concentrations (5, 20 and 30 μg ml^{-1}) each day in three different days (reproducibility) by both procedures. The method precision was established by assaying six replicates of authentic sample with the two proposed methods.

For the HPLC method, the relative standard deviations (R.S.D.) for the standard solutions were of 1.81% for repeatability and of 2.09, 2.19 and 2.84% for reproducibility at 5, 20 and 30 μg ml^{-1}, respectively, and of 3.17% for the sample.

The R.S.D. values obtained for the standard solutions by the UV first-derivative spectrophotometric procedure were of 1.53% for repeatability and of 1.63, 2.09 and 1.42% for reproducibility at 5, 20 and 30 μg ml^{-1}, respectively. The R.S.D. values for the sample was of 2.65%.

3.2. Linearity

Linearity of the response was determined for HPLC and first-derivative methods by preparing six standard solutions spanning 25%–150% of the amount expected (20 μg ml^{-1}). Linear regression analysis of the responses (y) (peak areas and absorbances for HPLC and spectrophotometric procedures, respectively), on the theoretical concentration (x) gave the equation $y = 80237.43x + 33817.02$ for the HPLC method and the equation

$y = 30.94 \times 10^{-5}x - 15.33 \times 10^{-5}$ for the first-derivative procedure. The determination coefficients were $r^2 = 0.9980$ for the HPLC method and $r^2 = 0.9988$ for the first-derivative procedure and confirmed the linearity of both methods over the concentration range analysed. The R.S.D. values of the slope and intercept of the LC method were 4.75 and 104.53%, while these values were 0.43 and −9.88% for the spectrophotometric procedure.

3.3. Accuracy

Nifedipine recoveries from placebo microemulsions were assessed by spiking placebo with nifedipine and following the same procedures that were used for the dosage form. Placebo was spiked in triplicate at three concentration levels spanning 50–150% of the amount of nifedipine in dosage form. The average nifedipine recoveries for the three levels studied was 97.78% for the HPLC method and 98.84% for first-derivative procedure with R.S.D. values of 1.82 and 1.68% for each method, respectively, (Table 2). Linear regression analysis of the dependence of the average amount recovered (y) on the average amount added (x) gave the equations $y = 0.97x + 0.17$ with a correlation coefficient (r^2) of 0.9982 for the HPLC method and $y = 0.98x + 0.01$ with a r^2 of 0.9993 for the spectrophotometric procedure.

3.4. Limit of detection (DL)

The calculated limits of detection were obtained from the following equation [27]:

$$DL = \sqrt{\frac{S_0^2 \times n - 2}{n - 1}} \times \frac{t_p}{b} \quad (1)$$

where n was the number of samples, t_p was the value of Student's t-test at $P = 0.05$ level of significance and (n-2) degrees of freedom, b was the slope and S_0^2 was the variance characterising the dispersion of the points regarding the regression line.

The experimental detection limits were established as the concentration where a significant difference could be seen between standard solution and spiked samples (paired Student's t-test, $P > 0.05$) F[28]. For their determination, the concentration of 20 μg ml^{-1} was selected as this concentration was the 100% of nifedipine standard sample.

The lowest experimental and calculated DL_s were for the first-derivative procedure with values of 0.5 and 0.39 μg ml^{-1}, respectively, while the experimental and calculated DL_s of the HPLC procedure presented values of 1 and 1.89 μg ml^{-1}, respectively. In spite of the fact that the detection limits for the first-derivative and HPLC methods were not statistically similar to those calculated according to (Eq. (1)), the calculated

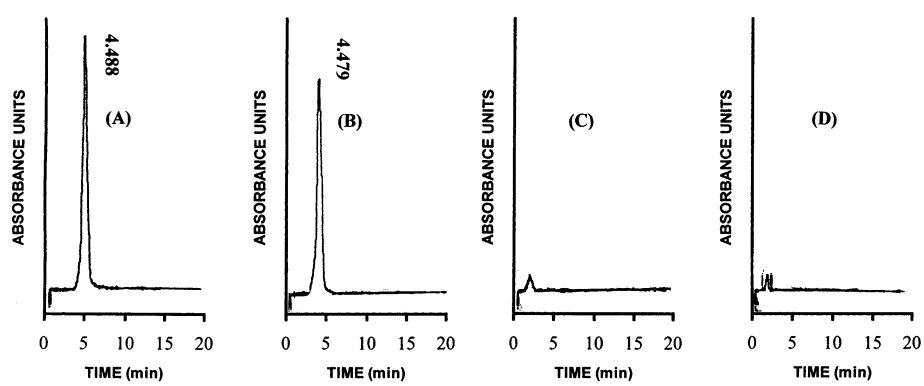

Fig. 4. HPLC chromatograms of (A) nifedipine loaded O/W/O multiple microemulsion (20 μg ml^{-1} nifedipine concentration in sample), (B) 20% decomposed nifedipine loaded O/W/O multiple microemulsion, (C) totally decomposed nifedipine loaded O/W/O multiple microemulsion and (D) placebo O/W/O multiple microemulsion.

Table 2
Recovery of nifedipine from spiked placebo O/W/O multiple microemulsions

$^1D_{387}$ assay			HPLC assay		
Amount added (mg)	Amount recovered (mg)	Recovery (%)	Amount added (mg)	Amount recovered (mg)	Recovery (%)
18.64	18.05	96.83	18.33	17.86	97.43
18.12	18.25	100.71	18.60	17.97	96.61
18.02	17.97	99.72	18.95	18.22	96.14
36.29	35.59	98.07	36.33	36.17	99.56
36.45	36.31	100.82	36.42	36.05	98.98
36.97	35.97	97.29	36.98	36.38	98.37
54.09	53.84	99.53	54.29	51.24	94.38
54.10	52.40	96.85	54.01	54.12	100.20
54.02	54.06	100.07	54.10	52.19	96.46
Mean	–	98.88	–	–	97.78
% R.S.D.	–	1.64	–	–	1.82

and experimental DL for both methods demonstrated that first-derivative procedure was much more sensitive than HPLC method.

3.5. Stability of samples assessed for the validation of the procedures

The stability of the sample solutions at 8, 25, 40 °C, 24 and 48 h after preparation was verified by re-assaying in order to study the stability of the samples throughout the validation. There was no indication of any decomposition of nifedipine in the samples analysed (Table 3) by the two analytical procedures described in this paper and so the samples were considered to be stable during all the analysis performed in this work since they were carried out at room temperature (approximately, 20 °C).

3.6. Specificity of the methods

In order to asses the specificity of the spectrophotometric and LC methods 5.55 ml of placebo O/W/O multiple microemulsion were transferred to a 100 ml volumetric flask and diluted to volume with methanol. This solution (1 ml) was pipetted to a 10 ml volumetric flask and diluted to volume with methanol.

Fig. 5(A) shows the zero order spectra of the excipients of the microemulsion. This spectra demonstrates that the overlapping of the excipients over the 200–280 nm region prevents us from noticing the interference of the drug decomposition products over the wavelength of 200–440 nm when it is compared with Fig. 2(A).

As it can be seen in Fig. 5(B), first-derivative spectrophotometric spectra showed no interference by the excipients used for the O/W/O multiple microemulsion formulation (Table 1) at the working wavelength ($\lambda = 387$ nm), since microemulsion excipients showed an absorption band at ~ 246 nm, and so the method was considered to be specific for the drug.

For the HPLC method the sample solution aforementioned was chromatographied and it was found to be specific for nifedipine since no peaks were recorded at the same retention time found for nifedipine Fig. 4(D), which meant that there was no interference of the formulation excipients in the determination of the drug.

3.7. Analysis of nifedipine microemulsions

In order to establish the proposed methods five nifedipine O/W/O multiple microemulsion were assayed for their recovery of partially decomposed nifedipine. The assays were carried out as described in Section 2.4. Table 4 shows nifedipine recovered from O/W/O multiple microemulsions without any decomposition and with different degrees of decomposed nifedipine. The partially decomposed drug recoveries obtained for both

Table 3
Stability samples determination results

Time/Condition	$^1D_{387}$ assay		HPLC assay	
	Amount found in multiple microemulsion (mg ml^{-1})[a]	Percent of initial concentration	Amount found in multiple microemulsion (mg ml^{-1})[a]	Percent of initial concentration
Initial	3.60 ± 0.01	–	3.60 ± 0.02	–
24 h/8 °C	3.58 ± 0.02	99.44	3.58 ± 0.03	99.44
24 h/25 °C	3.60 ± 0.02	100.00	3.62 ± 0.02	100.55
24 h/40 °C	3.53 ± 0.01	98.05	3.57 ± 0.04	99.17
48 h/8 °C	3.57 ± 0.02	99.16	3.55 ± 0.03	98.61
48 h/25 °C	3.56 ± 0.03	98.88	3.57 ± 0.02	99.16
48 h/40 °C	3.53 ± 0.01	98.05	3.54 ± 0.03	98.33

[a] Mean of three determinations ± S.D.

methods were in good agreement with the real contents of nifedipine without any decomposition achieved with these procedures.

4. Conclusion

As it has been mentioned earlier, direct spectrophotometry and second derivative spectrophotometry were not suitable for the quantitation of nifedipine in the presence of its decomposition products since both methods showed interferences with such products and even more the second-derivative procedure did not exhibit a good linear relationship between spectrophotometric responses and nifedipine concentrations. Nevertheless, first-derivative and HPLC methods were found to be linear, reproducible and capable of quantifying nifedipine without interference of its decomposition products and the excipients of the formulation. For both methods, no extraction procedures were needed, and hence they allowed the simple, fast and reliable quantitative analysis of the drug, which is always useful for routine determination.

Since, first-derivative spectroscopy procedures are less time consuming, less expensive and require less operational training than the HPLC methods and as far as this procedure gives better recoveries of the drug from O/W/O multiple microemulsions and presents more precision than the HPLC method described, we do recommend the first-derivative spectroscopy method for the quantitation of nifedipine in the presence of its decomposition products and the excipients used

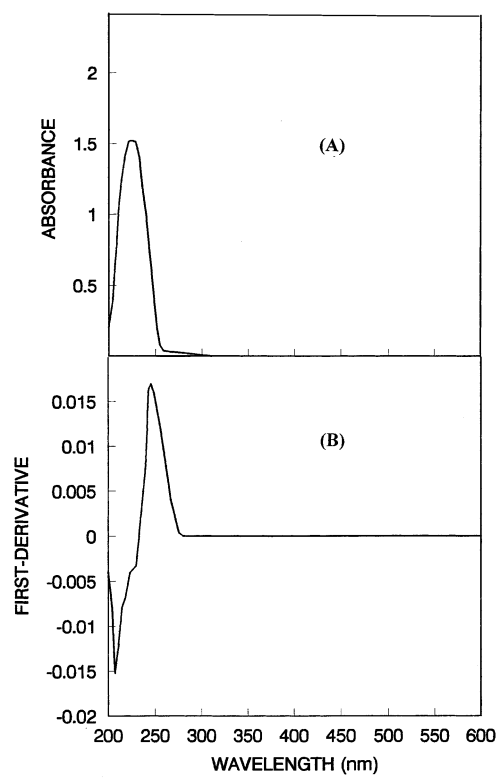

Fig. 5. (A) Zero order spectra of excipients placed in the O/W/O multiple microemulsion formulation; (B) First-derivative spectra of excipients placed in the O/W/O multiple microemulsion formulation.

Table 4
Results of assay of nifedipine and partially decomposed nifedipine in multiple microemulsion at 50 °C

Percentage of decomposed nifedipine	Decomposition time (days)	Nifedipine recovered (Mean ± S.D.) (%)[a]	
		$^1D_{387}$	HPLC
0	–	99.84 ± 0.16	100.0 ± 70.58
20	5	80.12 ± 0.19	80.0 ± 40.21
35	7	65.25 ± 0.34	64.93 ± 0.23

[a] Mean of five determination ± S.D.

for such disperse systems, as well as in bulk material, during stability studies.

References

[1] R.M. Robertson, D. Robertson, in: J.G. Hardman, L.E. Limbird (Eds.), Goodman and Gilman's the Pharmacological Basis of Therapeutics, Ninth ed., Pergamon Press, New York, 1996, pp. 749–763.
[2] J.A. Oates, in: J.G. Hardman, L.E. Limbird (Eds.), Goodman and Gilman's the Pharmacological Basis of Therapeutics, 9th edn, Pergamon Press, New York, 1996, pp. 764–783.
[3] V. Saano, P. Raatikainen, H. Paronen, R. Komulainen, Int. J. Clin. Pharmacol. Res. 9 (1989) 247–254.
[4] R.J. Weiss, Am. Fam. Phys. 44 (1991) 2075–2082.
[5] D.G. Waller, A.G. Renwick, B.S. Gruchy, C.F. George, Br. J. Clin. Pharmacol. 18 (1984) 951–954.
[6] C.H. Kleinbloesen, P. van Brummelen, J.A. van de Linde, P.J. Voogd, D.D. Breimer, Clin. Pharmacol. Ther. 35 (1984) 742–747.
[7] F.A. Tucker, P.S.B. Minty, G.A. Mcgregor, J. Chromatogr. 342 (1985) 193–198.
[8] W. Snedden, P.G. Fernandez, C. Nath, Can. J. Physiol. Pharmacol. 64 (1986) 290–296.
[9] I.A. Majeed, W.J. Murray, D.W. Newton, S. Othman, W.A. Al-Turk, J. Pharm. Pharmacol. 39 (1987) 1044.
[10] S. Kondo, A. Kuchiki, K Yamamoto, K. Akimoto, K. Takahashi, N. Awata, I. Sugimoto, Chem. Pharm. Bull. 28 (1980) 1–7.
[11] S.R. Hamann, R.G. McAllister Jr, Clin. Chem. 29 (1983) 158–160.
[12] M.E. Sheridan, G.S. Clark, M.L. Robinson, J. Pharm. Biomed. Anal. 7 (1989) 519–522.
[13] C.H. Kleinbloesen, P. van Harten, P. van Brummelen, D.D. Breimer, J. Chromatogr. 308 (1984) 209–216.
[14] K. Miyazaki, N. Kohri, T. Arita, H. Shimono, K. Katoh, A. Nomura, H. Yasuda, J. Chromatogr. 310 (1984) 219–222.
[15] A. Jankowski, H. Lamparczyk, J. Chromatogr. A 668 (1994) 469–473.
[16] V. Horváth, A. Hrabeczy-Pall, E. Niegreisz, E. Kocsi, G. Horvai, L. Godorhazy, A. Talokan, Y. Klebovich, K. Balogh-Nemes, J. Chromatogr. B. 686 (1996) 211–216.
[17] H. Suzuki, S. Fujiwara, S. Kondo, I. Sugimoto, J. Chromatogr. 341 (1985) 341–347.
[18] A. Syed Laik, in: K. Florey (Ed.), Analytical Profiles of Drug Substances, vol. 18, Academic Press, London, 1989, pp. 221–288.
[19] USP 24-NF 19, United States Pharmacopeial Convention, Inc., 12601 Twinbrook Parkway, Rockville, MD, 2000, pp. 1183-1184.
[20] A.F. el Walily, J. Pharm. Biomed. Anal. 16 (1997) 21.
[21] A.F. el Walily, Acta Pharm. Hung. 6 (1997) 89.
[22] J.S. Grundy, R. Kherani, R.T. Foster, Chromatogr. J. B 654 (1994) 146–151.
[23] M.A. Moreno, P. Frutos, M.P. Ballesteros, J.L. Lastres, D. Castro, Chem. Pharm. Bull. 48 (2000) 1623–1627.
[24] M.A. Moreno, P. Frutos, M.P. Ballesteros, Chromatographia 48 (1998) 803.
[25] J.H.M. Schellnes, I.M.M. van Haelst, J.B. Houston, D.D. Breimer, Xenobiotica 21 (1991) 547–553.
[26] T. Ohkubo, H. Noro, K. Sugawara, J. Pharm. Biomed. Anal. 10 (1992) 67–70.
[27] J.N. Miler, Analyst 116 (1991) 3–14.
[28] D. Castro, M.A. Moreno, S. Torrado, J.L. Lastres, J. Pharm. Biomed. Anal. 21 (1999) 291–298.