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# Comparison of UV spectrophotometric and LC methods for the determination of nortriptyline hydrochloride in polysorbate 80 based oil/water (o/w) microemulsions

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**Abstract**

A new rapid, reliable and specific UV spectrophotometric method was developed for the determination of nortriptyline hydrochloride formulated into o/w microemulsions. The UV spectra of nortriptyline standard solution in methanol and placebo (microemulsion without nortriptyline) were recorded over the wavelength range 200–600 nm and the spectra for placebo and nortriptyline loaded microemulsion were recorded over the range 260–400 nm in order to determine the overlapping that might appears, and hence to set the wavelength that could be used for the quantitative analysis. 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 RSD values of 2.37 and 1.41%, respectively, for the LC method, and values of 1.24 and 2.88%, respectively, for the UV spectrophotometric method. The established linearity range was 10–50 μg $ml^{-1}$ ($r^2 = 0.9985$) and 20–60 μg $ml^{-1}$ ($r^2 = 0.9979$) for the HPLC and UV spectrophotometric methods respectively. The recoveries of nortriptyline from spiked placebos were $> 95\%$ for both methods over the linear range. The methods have been successfully used for determining the nortriptyline content of microemulsions and for evaluating the chemical stability of the drug in nortriptyline-loaded microemulsions. 

*Keywords:* High performance liquid chromatography; UV absorption spectroscopy; Oil/water microemulsions; Nortriptyline hydrochloride

## 1. Introduction

Nortriptyline hydrochloride [3-(10,11-Dihydro-5H - dibenzo[a,d]cyclohepten - 5 - ylidene)propyl-(methyl)amine hydrochloride] (Fig. 1), is a tricyclic antidepressant and the main active metabolite of amitriptyline which has a molecular weight of 299.8. It has been widely used, due to its high in vivo activity, in the treatment of major depressive disorders [1–3], pain of varying ethiology [4], nocturnal enuresis [5], pathological crying or laughing [6] and tinnitus [7].

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Many methods have been described in literature for the determination of nortriptyline and other tricyclic antidepressants in biological samples, such as plasma and urine, using fluorescence polarisation immunoassay [8], chromatographic procedures [9–13], capillary zone electrophoresis [14] and colloidal metal immunoassay [15].

Although immunological techniques seem to be, in general terms, more sensitive, they have many disadvantages that include the time consuming ( $\sim$ 24 h) to carry out the assays, the large number of steps in procedure, and the need to develop antiserum [8].

Since most of the chromatographic methods developed have been used in pharmacokinetic investigations, special detectors, such as mass spectrometry [10], nitrogen phosphorous [13,16], were required in order to quantify low concentrations of nortriptyline in biological fluids.

In the last 8 years the only method found in literature that quantifies nortriptyline in pharmaceutical dosage forms was the HPLC method proposed by the USP 23 [17] for the uniformity of content determination in nortriptyline capsules, and because of this, a novel and specific UV spectrophotometric method has been developed and compared with an RP–HPLC method described by Kabra et al. [18], for the determination of nortriptyline within a new transdermal dosage form, o/w microemulsions, carried out by our research group [19] in order to diminish the high first pass effect suffered by the drug when it is administered orally [20]. This new analytical procedure was able to accurately quantify the drug in the presence of the formulation excipients without any need of using any special detectors or other much more time consuming analytical techniques.

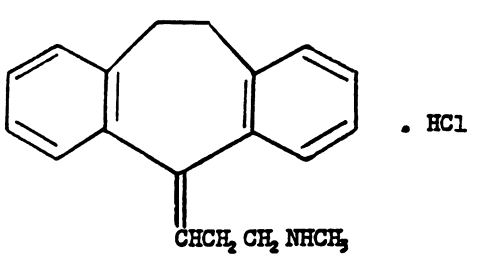


Fig. 1. Chemical structure of nortriptyline hydrochloride.

## 2. Experimental

### 2.1. Chemicals

Nortriptyline hydrochloride was a gift from Synthelabo S.A. (Madrid, Spain); microemulsion components were isoprpyl myristate (Merck Chemicals, Barcelona, Spain), propylene glycol (Panreac, Madrid, Spain), Polysorbate 80 (Sigma Chemicals, Madrid, Spain); all other reagents used were HPLC grade and provided by Sigma Chemicals (Madrid, Spain). The water used for both microemulsion and buffer preparations was deionised and was obtained from a Milli-Q Plus system (Millipore, Madrid, Spain).

### 2.2. Apparatus and conditions

Two different absorption spectra were carried out using methanol as a blank, (a) one for nortriptyline standard solution in methanol (30 $\mu g \cdot ml^{-1}$), placebo microemulsion (microemulsion without nortriptyline) in methanol at a concentration of 0.005% (v/v) and polysorbate 80 in methanol at a concentration of approximately 2 $\mu g \cdot ml^{-1}$, recorded over the wavelength range 200–600 nm in order to identify absorption maximums and (b) for placebo and nortriptyline-loaded microemulsion (6 mg $ml^{-1}$) in methanol at a concentration of 0.005% (v/v) for both samples, recorded over the range 260–400 nm in order to determine the overlapping that might occurs between the drug and the dosage form on the absorption maximums that were found, and therefore to set the wavelength used for the quantitative analytical spectrophotometric method.

For this purpose, a 1 cm quartz cells and a Beckman DU®-7 spectrophotometer with a scan speed of 300 nm $min^{-1}$ were used to obtain both spectra.

The absorption wavelength set for all the spectrophotometric measurements was of 267 nm, as it was concluded from the different spectra studied.

The HPLC method used was based on the method developed by Kabra et al. [18] with some modifications related to and mobile phase composition which gave chromatographic responses at

Table 1
Composition (% w/w) of placebo and nortriptyline microemulsions

| | Placebo | Nortriptyline microemulsion |
|---|---|---|
| *Drug* | | |
| Nortriptyline | – | 0.6 |
| *Excipients* | | |
| Isopropyl myristate | 7.5 | 7.5 |
| Phosphate buffer (pH 7.4) USP 23 | 46.3 | 46.3 |
| Polysorbate 80 | 34.3 | 34.3 |
| Propyleneglycol | 11.3 | 11.3 |

earlier retention times (about 6 min) than the ones obtained in the original procedure (about 9 min).

Since this method was able to quantify of the drug in the presence of the formulation excipients, it was used to compare the results obtained with the spectrophotometric 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 239 nm (nortriptyline absorption maximum), were used. The 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 ODS-Hypersil Column of $200 \times 4.6$ mm ID and 5 μm particle size (Teknokroma Madrid, Spain). The mobile phase was a mixture of phosphate buffer (pH 3) and acetonitrile (65:35, v/v), filtered through 0.45 μm nylon filters, degassed and pumped at a constant flow rate of 2 ml $min^{-1}$. To prepare the phosphate buffer, 0.6 ml of nonylamine were added to 1000 ml of 0.01 M sodium dihydrogen phosphate and adjusted the pH to 3 by the addition of phosphoric acid. The injection volume was 20 μl for all standards and samples.

### 2.3. Standard preparations

For the spectrophotometric method, 50 mg of nortriptyline and 10 ml of placebo microemulsion (composition shown in Table 1) were accurately measured, transferred to a 50 ml volumetric flask and diluted to volume with methanol to obtain a nortriptyline concentration of 1 mg $ml^{-1}$. Further dilutions were made with methanol to obtain a range of concentration between 20 and 60 μg $ml^{-1}$.

For the HPLC method, 50 mg of nortriptyline were accurately weighed, transferred to a 50 ml volumetric flask and diluted to volume with methanol to yield a nortriptyline concentration of 1 mg $ml^{-1}$. Further dilutions were also made with methanol to obtain a concentration range 10–50 μg $ml^{-1}$. The differences in the concentration range were due to the drug absorbances, which were low in the spectrophotometric method because of the blank employed, and so it was not possible to measure the absorbances of concentrations lower than 20 μg $ml^{-1}$.

### 2.4. Sample solutions

For both analytical methods, 10 ml of nortriptyline loaded microemulsion (composition shown in Table 1) containing 60 mg of such active ingredient, were measured, transferred to a 200 ml volumetric flask and diluted to volume with methanol. This solution (1 ml) was pippeted to a 10 ml volumetric flask and diluted to volume with methanol to yield a final nortriptyline concentration of 30 μg $ml^{-1}$.

### 2.5. Calibration

Aliquots of the standard stock solution of nortriptyline were pipetted into different 100 ml volumetric flasks and diluted to volume with methanol. The final concentrations of nortriptyline were in the range 10–50 μg $ml^{-1}$ for the HPLC procedure and 20–60 μg $ml^{-1}$ for the spectrophotometric method. Each solution was analysed in triplicate for both methods. Peak areas were recorded at 239 nm and absorbances were recorded at 267 nm for each procedure, respectively.

### 2.6. System suitability test

For the HPLC method, the system suitability 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 of variation was $< 5\%$ and the tailing factor $< 1.5\%$.

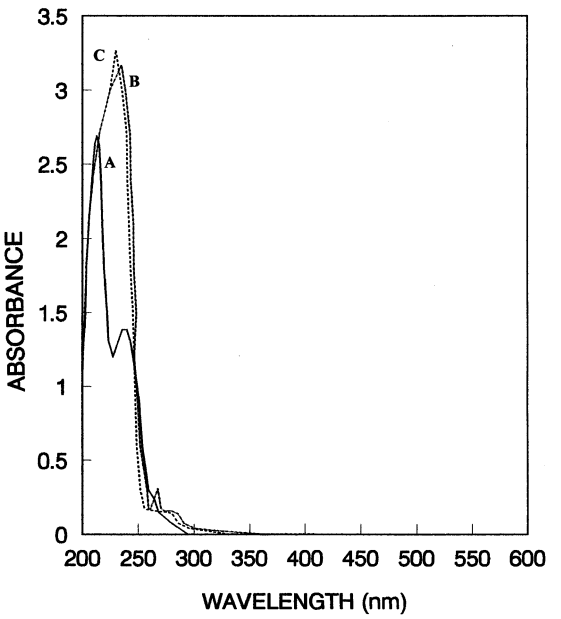


Fig. 2. Absorption zero-order spectra of (A) (—) nortriptyline chlorhydrate standard solution in methanol (30 μg ml$^{-1}$), (B) (— · —) placebo microemulsion in methanol (0.005%, v/v), and (C) (.....) polysorbate 80 in methanol ( ≈ 2 μg ml$^{-1}$).

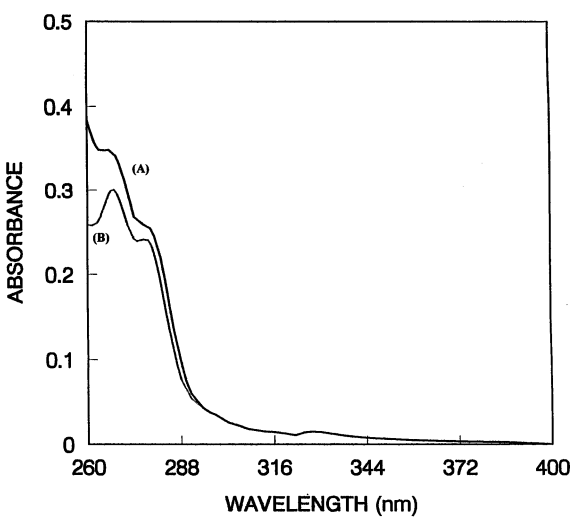


Fig. 3. Absorption zero-order spectra of (A) (—) nortriptyline loaded microemulsion (6 mg ml$^{-1}$) in methanol (0.005%, v/v) and (B) (·····) placebo microemulsion in methanol (0.005%, v/v).

### 2.7. Procedure

For the HPLC method, six injections of standard and three injections of sample preparation (each 20 μl) were chromatographed. For the spectrophotometric method six measures of the standard solutions and three of sample preparations were carried out. The quantity of nortriptyline ml$^{-1}$, in both methods, was calculated by the following formula

Amount of nortriptyline (mg ml$^{-1}$ microemulsions) $= (R_{sam}/R_{std})C \times F \times 10^{-3}$.

Where $R_{sam}$ and $R_{std}$ are the average peak responses and the absorbances of sample preparation and standard preparation, respectively, in both methods, $C$ the concentration (μg ml$^{-1}$) of the standard preparation and $F$ is the dilution factor.

## 3. Results and discussion

Fig. 2 shows the zero order spectra of nortriptyline hydrochloride standard solution (A) placebo microemulsion (B) and polysorbate 80 (C). As it can be seen, there was considerable overlapping between the three spectra at the maximum absorption wavelength for nortriptyline (239 nm) practically due to the presence of such surfactant on the microemulsion composition (see Fig. 2) since the rest of the components of the dispersed system showed no response at the concentrations used (data not shown).

This fact prevent the aforementioned wavelength from its use as a set parameter for the development of the spectrophotometric method. However, when the spectra of placebo and nortriptyline-loaded microemulsion were compared (see Fig. 3), there was an amplitude between both spectra due to nortriptyline absorption, which reached its highest difference at the wavelength of 267 nm. This meant that such wavelength could be used, and so it was done, for the quantitation of the drug, using a placebo solution as a blank, since it gave the best linear response, a near zero

intercept of the ordinate of the calibration and is the least affected by the excipients used in the formulation. Neither first nor second derivative spectra were suitable for overcoming this problem, as they were not able to accurately quantify nortiptyline from dosage forms of this kind.

The proposed chromatographic and UV spectrophotometric methods were assessed for specificity, linearity, precision, accuracy and stability.

### 3.1. Precision

The system precision was determined for the HPLC method by chromatographing six injections of the standard within the same day (repeatability) and three injections of the standard each day in three different days (reproducibility). The method precision was established by assaying six replicates of authentic sample with the proposed method. The relative standard deviations (RSD) for the standard were of 2.37 and 3.58% for repeatability and reproducibility, respectively, and of 2.45% for the sample.

The system and method precision for the spectrophotometric method were determined as above, by measuring the spectrophotometric responses of standard and sample preparations. The RSD for the standard were of 1.24 and 1.71% for repeatability and reproducibility, respectively, and of 2.16% for the sample.

### 3.2. Linearity

The linearity of the response was determined for the HPLC method by chromatographing five standard solutions spanning 35–165% of the amount expected (30.0 $\mu g \cdot ml^{-1}$). For the spectrophotometric procedure, the linearity was assessed by measuring the absorbances of five standard solutions spanning 65–200% of the amount expected (30.0 $\mu g \cdot ml^{-1}$). Linear regression analysis of the responses ($y$) (peak areas and absorbances for HPLC and spectrophotometric methods, respectively) on the theoretical concentration ($x$) gave the equation $y = 192938.78x + 169857.90$ for the HPLC method and the equation $y = 0.00423x + 0.0008$ for the spectrophotometric procedure. The determination coefficients, $r^2 = 0.9985$ for the HPLC method, and $r^2 = 0.9979$ for the spectrophotometric procedure, confirmed the linearity of both methods over the concentration range analysed. The RSD values of the slope and the intercept of the LC method are 0.62 and 30.61%, respectively, while these values are 1.11 and 6.20% for the spectrophotometric method.

### 3.3. Accuracy

The recoveries of nortriptyline from placebo microemulsions were assessed by spiking placebo with nortriptyline and following the same procedures that were used for the dosage form. Placebo was spiked in triplicate at three levels spanning 50–150% of the amount of nortriptyline in the dosage form. The average recovery for the three levels was 99.6% for the HPLC method and 95.4% for the spectrophotometric procedure with % RSD values of 1.1 and 2.9% 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 $y = 0.99x + 0.36$, with a correlation coefficient of 0.9993 for the HPLC method and $y = 0.90x + 2.69$ with a correlation coefficient 0.9984 for the spectrophotometric procedure.

### 3.4. Stability of samples

The stability of the sample solutions at 8, 25 and 40°C, 24 and 72 h after preparation was verified by re-assaying in order to asses the stability of the samples throughout the validation. There was no indication of any decomposition of nortriptyline chlorhydrate in the samples analysed (Table 3) by the two analytical procedures described in this paper, and because of this, 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).

### 3.5. Specificity of the spectrophotometric method

Since the spectrophotometric method was developed with the absorbances of the samples against a blank previously described (placebo mi-

Table 2
Recovery of nortriptyline hydrochloride from spiked placebo microemulsions

| UV spectrophotometric assay | | | HPLC assay | | |
|---|---|---|---|---|---|
| Amount added (mg) | Amount recovered (mg) | Recovery (%) | Amount added (mg) | Amount recovered (mg) | Recovery (%) |
| 30.50 | 28.39 | 93.09 | 30.50 | 30.78 | 100.92 |
| 29.90 | 29.57 | 98.91 | 30.20 | 30.07 | 99.57 |
| 30.10 | 29.57 | 98.25 | 30.20 | 30.49 | 100.96 |
| 60.30 | 58.20 | 96.52 | 60.05 | 58.56 | 97.52 |
| 60.00 | 58.73 | 96.22 | 59.98 | 59.28 | 98.83 |
| 60.10 | 59.15 | 98.42 | 60.89 | 61.23 | 100.56 |
| 90.20 | 83.05 | 92.08 | 90.00 | 89.91 | 99.90 |
| 90.30 | 84.47 | 93.55 | 90.00 | 90.76 | 100.84 |
| 90.00 | 82.35 | 91.50 | 89.89 | 87.55 | 97.37 |
| Mean | | 95.39 | | | 99.61 |
| % RSD | | 2.88 | | | 1.41 |

Table 3
Results from determination of the stability of samples

| Condition/time | UV spectrophotometric assay | | HPLC assay | |
|---|---|---|---|---|
| | Amount declared in microemulsions (mg ml$^{-1}$)[a] | Percent of initial concentration | Amount declared in microemulsions (mg ml$^{-1}$)[a] | Percent of initial concentration |
| Initial | $2.49 \pm 0.02$ | – | $2.53 \pm 0.03$ | – |
| 24 h/8°C | $2.44 \pm 0.02$ | 97.99 | $2.54 \pm 0.02$ | 100.39 |
| 24 h/25°C | $2.47 \pm 0.02$ | 99.19 | $2.55 \pm 0.03$ | 100.79 |
| 24 h/40°C | $2.46 \pm 0.02$ | 98.79 | $2.56 \pm 0.02$ | 101.18 |
| 72 h/8°C | $2.42 \pm 0.01$ | 97.36 | $2.46 \pm 0.02$ | 97.23 |
| 72 h/25°C | $2.42 \pm 0.01$ | 97.19 | $2.46 \pm 0.01$ | 97.23 |
| 72 h/40°C | $2.40 \pm 0.01$ | 96.38 | $2.45 \pm 0.02$ | 96.83 |

[a] Mean $\pm$ standard deviation ($n = 3$).

croemulsion), it was not necessary to asses its specificity.

### 3.6. Specificity of the HPLC method

In order to asses the specificity of the HPLC method, 10 ml of placebo microemulsion were transferred to a 200 ml volumetric flask and diluted to volume with methanol. This solution (1 ml) was pipetted to a 10 ml volumetric flask, diluted to volume with methanol and chromatographied. The method was found to be specific for nortriptiline as no peaks were recorded at same retention time of the peak recorded for the drug, as it can be seen in Fig. 4.

### 3.7. Analysis of nortryptiline microemulsions

In order to establish the proposed methods, five nortriptyline microemulsions were assayed with both methods in duplicate. The assayed values are presented in Table 4. There are practically no differences between the amount declared and the amount found for each microemulsion.

## 4. Conclusions

The described methods were found to be linear, reproducible, accurate and capable of quantifying

nortiptyline chlorhydrate in the presence of the microemulsion excipients. Neither the spectrophotometric nor the HPLC methods required any extraction procedure, and hence they allowed the simple, fast and reliable quantitative analysis of the drug, which is always useful for routine determinations.

Since spectrophotometric procedures, are less time consuming, less expensive and requires less operational training than the HPLC methods, we

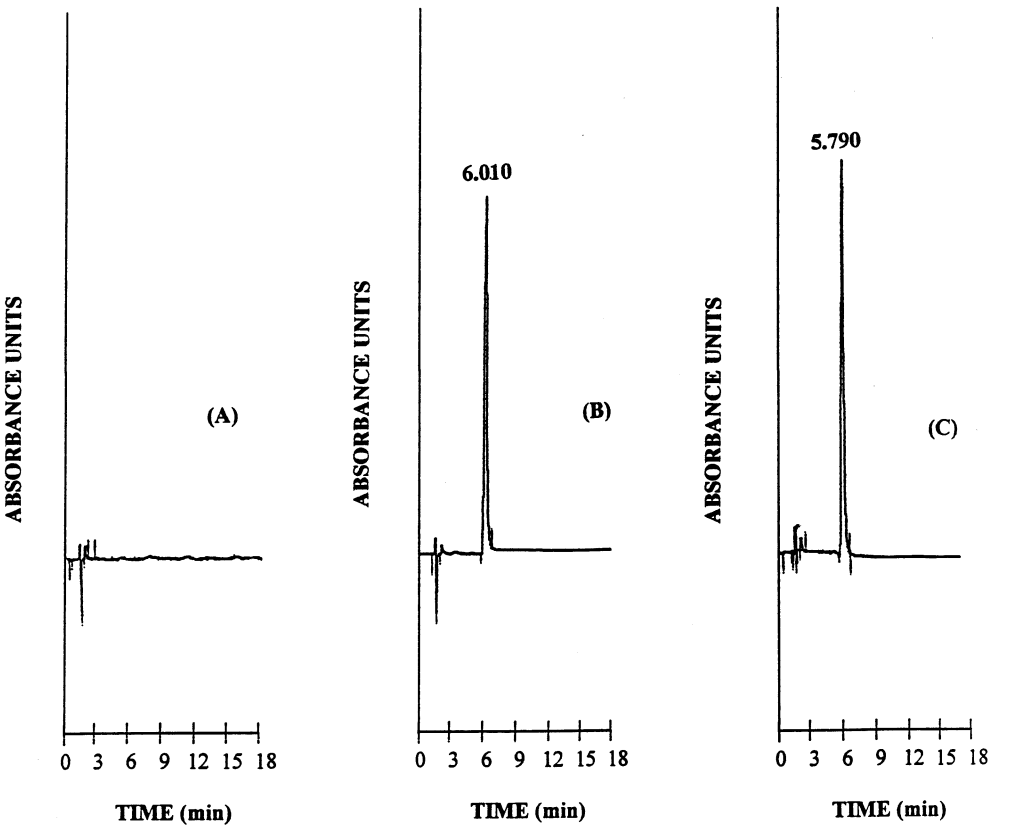


Fig. 4. Chromatograms and retention times (minutes) of (A) placebo microemulsion, (B) nortriptyline hydrochloride standard solution (30 μg $ml^{-1}$), and (C) nortriptyline loaded microemulsion (30 μg $ml^{-1}$ nortriptyline concentration in sample).

Table 4
Results of assay of nortriptyline hydrochloride in microemulsions

| Microemulsions | UV Spectrophotometric assay | | HPLC assay | |
|---|---|---|---|---|
| | Amount declared (mg $ml^{-1}$) | Amount found (mg $ml^{-1}$) | Amount declared (mg $ml^{-1}$) | Amount found (mg $ml^{-1}$) |
| A | 2.50 | 2.46 | 2.50 | 2.51 |
| B | 2.50 | 2.49 | 2.50 | 2.52 |
| C | 2.50 | 2.48 | 2.50 | 2.50 |
| D | 2.50 | 2.47 | 2.50 | 2.49 |
| E | 2.50 | 2.47 | 2.50 | 2.47 |

can highly recommend the new spectrophotometric method developed in this work as it was much more precise than the HPLC procedure and might be used to quantify nortriptiline hydrochloride in any dispersed system containing polisorbate 80 in its composition.

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