Sensitive Extractive Spectrophotometric Methods for the Determination of Nortriptyline Hydrochloride in Pharmaceutical Formulations

Wieslawa MISIUK* and Agnieszka TYKOCKA

Department of Biology and Chemistry, Institute of Chemistry, University of Bialystok; 15–399 Bialystok, Poland.

Two simple, sensitive and rapid extractive spectrophotometric methods have been developed for the assay of the antidepressant drug nortriptyline (NOR) hydrochloride in pure form and in different dosage forms. The methods involve the formation of colored ion-pairs between the drug and the complex of niobium(V)-thiocyanate (Nb-SCN) or iron(III)-thiocyanate (Fe-SCN) followed by their extraction with butanol or a mixture of butanol and chloroform and quantitative determination at 360 nm and 490 nm, using Nb-SCN and Fe-SCN, respectively. The experimental conditions were optimized to obtain the maximum colour intensity. The methods permit the determination of nortriptyline over a concentration range of 15—100 μg/ml and 5—24 μg/ml with the detection limit of 0.84 μg/ml and 0.32 μg/ml, using Nb-SCN and Fe-SCN, respectively. The proposed methods are applicable for the assay of the investigated drug in different dosage forms and the results are in good agreement with those obtained by the official and HPLC methods. No interference was observed from common excipients present in pharmaceutical formulations. The proposed procedures were applied to determine the amount of nortriptyline hydrochloride as active ingredient in the presence of its degradation product, dibenzosuberone. The extractive spectrophotometric methods can also be used to determine the amount of nortriptyline hydrochloride in tablets after its solid phase extraction (SPE).

Key words spectrophotometric determination; nortriptyline hydrochloride; degradation product; solid phase extraction; pharmaceutical preparation

Nortriptyline (NOR) belongs to the family of tricyclic antidepressants, a group of drugs widely used for treating depressive diseases. Its chemical structure is shown in Fig. 1.

Most existing methods for the determination of nortriptyline in bulk, pharmaceutical formulations and biological samples have been reviewed.[1] Among other methods described for the assay of this drug are direct UV-spectrophotometry,[2,3] derivatives spectrophotometry,[4,5] fluorimetry[6] and colourimetry.[7,8] The colourimetric methods involve ion association complex with dye—Light Green FCF at pH 5 and Orange II[7] at pH 2 or charge-transfer complex with 7,7,8,8-tetracyanoquinodimethane (TCQN).[8]

Extractive spectrophotometric procedures are popular for their sensitivity in the assay of drugs and, therefore, ion-pair extractive spectrophotometry has received considerable attention for the quantitative determination of many pharmaceutical compounds.[9—16]

Fig. 1. Molecular Structure of Nortriptyline (A) and Dibenzosuberone (B)

Quantitative analysis of the drugs applying extractive spectrophotometric methods is mainly founded on such factors as type of solvent extraction, nature of coordination agents, acidity of the solution, concentration of reagents, temperature, time of reaction and extraction.

Some methodological reports for the determination of nortriptyline[6,7,11,21,25—27] using specrophotometry have been published. However, these methods have several disadvantages in terms of cost performance, consumption of time and essential equipment, difficult reaction conditions and procedures for determination of drugs.

For example, De La Pena *et al.*[6] developed a fast kinetic method for the determination of nortriptyline in pharmaceutical preparations by use of photometric and fluorimetric detection. But the applied procedures required a temperature of 50 °C for both types of detection. Obtained linear dynamic ranges of the drug determinations were too broad.

Another method[21] was based on oxidation of imipramine and desipramine (drugs related to nortriptyline) with ammonium metavanadate. The method has been developed for determination of the drugs in the visible (VIS) range. However, the applied oxidizing agent cannot be used for determination of nortriptyline in the VIS range. The drug oxidation product is colourless and ammonium metavanadate is an unsuitable agent for its production.

Oztunc *et al.*[8] have proposed a method for nortriptyline determination which exhibited disadvantages in terms of difficult reaction conditions. In this procedure maximum absorption was reached at 80 °C and heating time of 10 min was recommended for maximum intensity colour development.

Most of the proposed agents for spectrophotometric determination of nortriptyline and related drugs are unsatisfactory for different reasons, *e.g.*, a long period for maximum intensity color development, lengthy and difficult procedure, lack
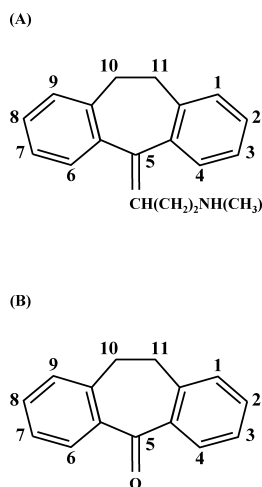
of sensitivity and specificity.

The literature on extractive spectrophotometric methods for the assay of nortriptyline is scanty, although the methods are suited for the drug's determination in a substance and pharmaceuticals. Extraction of the drug content from the powdered tablets or capsules is useful for eliminating the common expicients in drug formulations.

In order to continue our work on psychotropic drugs analysis[17—21] the aim of the present work is to utilise simple, reliable and accurate extractive spectrophotometric methods for the determination of the antidepressant drug, nortriptyline in pure form and in different pharmaceutical formulations available on the Polish markets. The methods are based on the reaction between this drug with niobium(V) and iron(III) thiocyanate complexes. The structures of the formed ion-pairs were examined. The methods developed were used to determine the quantity of nortriptyline in its pure form as well as in the presence of its degradation product—dibenzosuberone (Fig. 1). The results of the analysis were validated by statistical methods[22—24] and recovery studies. Common additives used as excipients in pharmaceutical dosage forms do not interfere in the determination of nortriptyline hydrochloride by the proposed methods. Extractive spectrophotometric methods can also be developed to determine the quantity of nortriptyline hydrochloride after its isolation from tablets by solid phase extraction (SPE).

Experimental

Reagents All chemicals were of analytical reagent grade and solvents were always HPLC or spectroscopic grade. Doubly distilled water was used to prepare all solutions. Freshly prepared solutions were always employed.

Pharmaceutical grade nortriptyline hydrochloride (Sigma) was used as a working standard. A stock standard solution of 0.001 mol/l of the drug was prepared by dissolving nortriptyline in doubly distilled water. Working standard solutions were then prepared by suitable dilution of the standard stock solution with water.

Apparatus A CECIL 8020 UV–VIS spectrophotometer with 1 cm quartz cells was used for all absorbance measurements. Spectra were automatically obtained by CECIL UV system software. Infrared spectra were obtained in the range 400—4000 cm^{-1} using an FT-IR Magna 550 II series, Nicolet spectrometer.

The chromatographic system, (Thermo Separation) consisted of a 3D detector Spectra System UV 3000, a low-gradient pump P2000, a vacuum membrane degasser SCM Thermo Separation and a Rheodyne loop injector (20 μl). The detector was set at 230—252 nm using the reversed phase analytical column, Lichrospher 100 RP-18 250×4 (5 μl) with a guard column 4×4 mm (5 μl) (Merck, Germany). ChromQuest Chromatography Data System software version for Windows NT was used for the acquisition and storage of data.

Preparation of Degradation Product of Nortriptyline Into a separate flask 0.3 g of nortriptyline hydrochloride was dissolved in 25 ml distilled water and 2 ml of 30% hydrogen peroxide was added. The solution was boiled to drive off excess oxygen, putting a funnel on top of the flask to minimise evaporation, and then cooled. One hour was sufficient for the reaction to go to completion and to drive off excess oxygen. The course of the reaction was followed up by TLC using silica gel 60 GF_{254} TLC plates and a mobile phase of benzene : methanol : ammonia (9 : 1 : 0.1). The solution was quantitatively transferred into a 50 ml volumetric flask and water added to the mark. The solution was used for laboratory prepared mixtures. The included degradation product (dibenzosuberone) in nortriptyline hydrochloride was tested with saturated sodium bisulfite which resulted in the formation of a white precipitate indicating the presence of the C=O group and confirmed by IR spectroscopy. This degradation product was found to have mp 32—34 °C. The material was tested for complete degradation using the HPLC and TLC systems described above. Using the HPLC system a single peak at a retention time of 14.23 min for dibenzosuberone was observed, while no peak was observed at a retention time of 3.85 min corresponding to intact nortriptyline hydrochloride. Using the TLC system, a single spot of Rf

0.80 was observed while none was observed at Rf 0.68 corresponding for nortriptyline.

Laboratory Prepared Mixtures Mixtures containing different ratios of nortriptyline hydrochloride and its laboratory prepared degradation product were studied and found to contain 10—70% of degradation product.

Procedures. Determination of Nortriptyline Hydrochloride Into a series of 50 ml separating funnels 5—100 μg/ml of standard drug solution, 2 ml of 10 mol/l $HClO_4$ for niobium(V)-thiocyanate (Nb-SCN) method (A) or 1.5 ml of 10 mol/l H_2SO_4 for iron(III)-thiocyanate (Fe-SCN) method (B), 2 ml (A) or 1 ml (B) of 10 mol/l KSCN, 2 ml of 10^{-2} mol/l Nb(V) or 3 ml of 10^{-2} mol/l Fe(III) were added and mixed well. The funnels were shaken vigorously for 2 min with 10 ml buthanol using Nb-SCN method or 10 ml of the buthanol–chloroform mixture (1 : 4, v/v) by Fe-SCN method and then allowed to stand for clear separation of the two phases. The separated organic phase was transferred to a 10 ml volumetric flask. Then the combined extract was made up to the mark with the solvent and mixed well. The absorbance of the organic phase was measured at 360 nm and 490 nm using Nb-SCN and Fe-SCN methods, respectively, against a reagent blank similarly prepared. The standard calibration plots were prepared to calculate the amount of the analyte drug in unknown samples. Colour of the extracts was stable for at least 3 h. All measurements were made at room temperature (25 °C±1 °C).

Procedure for Tablets and Capsules The total contents of the tablets or capsules were weighed and ground to a fine powder using a pestle and mortar. The average weight of a tablet or capsule was calculated. An accurately weighed portion of the powder equivalent to 25—75 mg of NOR was transferred into a 50 ml volumetric flask. The volume was made up to the mark with distilled water, shaken well, and filtered through ordinary filter paper. Convenient aliquots from this solution were taken for the determination of nortriptyline by Nb-SCN (range 15—100 μg/ml) and Fe-SCN (range 5—24 μg/ml) methods. The results were compared with those obtained by the pharmacopoeial method.[2] Table 5 summarizes the results.

Solid Phase Extraction (SPE) Before use the SPE column was properly conditioned as follows: a C_{18} extraction column was successively conditioned by 2.0 ml of methanol, 2.0 ml of water, and 1.0 ml of acetate buffer (pH 4.0; 0.1 mol/l). For this purpose the 1 ml cartridge C_{18} column was used. A 2 ml aliquot of the sample solution was applied to the SPE column.

Sample Preparation for SPE A sample equivalent to 1.0 mg of nortriptyline hydrochloride was dissolved in 100 ml of methanol and a 1.0 ml of aliquot of the sample solution was mixed with 1.0 ml acetate buffer (pH 4.0; 0.1 mol/l). After loading the whole spiked sample onto the activated column, the cartridge was then washed with 0.5 ml of a chloroform–acetone mixture (1 : 1, v/v). Afterwards, the column was dried completely before the eluting step. Elution was done with six 1.0 ml portions of the chloroform–n-butanol mixture (2 : 1, v/v) into a glass tube and then transferred to a 50 ml separatory funnel. Finally, the extract was subjected to extraction spectrophotometric determination according to the proposed procedures described in the section "Determination of Nortriptyline Hydrochloride."

Results and Discussion

Anionic thiocyanate complexes of niobium(V) and iron(III) formed ion associates with the positively charged drug. The stoichiometric ratios of NOR : Nb(V) or NOR : Fe(III) were studied by the continuous variation and mole-ratio methods and were found to be 2 : 1 for each associate. Each ion association complex, with two oppositely charged ions, behaved as a single unit held together by an electrostatic force of attraction.

Spectral Characteristics Absorption spectra of the yellow NOR–Nb-SCN and red NOR–Fe-SCN ion-pair compounds exhibited maximum at 360 and 490 nm, respectively. The colourless blanks had practically negligible absorbance.

Optimization of Variables Optimum conditions necessary for rapid and quantitative formation of coloured ion-pair complexes with maximum stability and sensitivity were established by a number of preliminary experiments. The effect of the extracting solvent on the ion-pair complexes was examined. A number of organic solvents such as butanol, chloroform, carbon tetrachloride, ether, dichloromethane and

their mixtures were studied for extraction of the complexes in order to provide an applicable extraction procedure. Butanol and a mixture of butanol–chloroform (1 : 4, v/v) using Nb-SCN and Fe-SCN methods, respectively, were preferred to other solvents, because of their higher efficiency in colour intensity, selective and quantitative extraction of the ion association complexes from the aqueous solutions and because they obtained the highest absorbance of coloured extracts. Comparison of absorbance within both organic and aqueous phases (before and after extraction) showed that in the case of butanol and the mixture of butanol–chloroform (1 : 4, v/v), the recovery of the extraction of ion-pair complexes was 100%, in both the NOR–Nb-SCN and NOR–Fe-SCN systems.

Ion-pair complexes were formed by using a magnetic stirrer and separating funnel. Shaking time ranging from 0.5 to 5 min provided a constant absorbance and hence, 2 min was used as an optimum shaking time throughout the experiments. The ion association complexes were quantitatively recovered in a single extraction and were also stable for at least 3 h.

To study the effect of heating time and temperature on reaction yield, we carried out the reaction at 25, 30 and 40 °C with heating time ranging from 1 to 30 min. It was found that the relative absorbance increased with time up 5 min at room temperature and when heated to 40 °C. The obtained results showed that the reaction of nortriptyline with tiocyanate complexes of niobium(V) and iron(III) was completed at room temperature (25 °C) and there was no need heating the reaction solutions.

The coloured ion-pair complexes developed in a few minutes after all the reagents had been added and mixed and attained maximum intensity after about 5 min at 25 °C. The absorbance of extracts was read after 1, 5, 10, 15 and 20 min and it was found that the absorbance could be read after 1 min as it had achieved the maximum and was constant. The analysis time required from sampling to measurement was about 7—10 min.

Optimum conditions were fixed by varying one parameter at a time while keeping other parameters constant and observing the effect on the absorbance at 360 and 490 nm by Nb-SCN and Fe-SCN methods, respectively. The effect of acid concentration was studied by extracting the coloured complex species at different ranges of acids. A maximal absorbance was observed in the range of acid concentration of 2—3 mol/l $HClO_4$ and 1.2—1.8 mol/l H_2SO_4 for NOR–Nb-SCN and NOR–Fe-SCN systems, respectively. A concentration of 2 mol/l $HClO_4$ and 1.5 mol/l H_2SO_4, respectively, were chosen as optimal using these systems. The effect of variation of thiocyanate and niobium(V) or iron(III) concentration should be studied in the presence of NOR hydrochloride. A maximal absorbance was observed in the range of 1.8—2.5 mol/l or 0.9—1.2 mol/l of potassium thiocyanate for NOR–Nb-SCN and NOR–Fe-SCN systems, respectively and of 1.2×10^{-3}—2.5×10^{-3} mol/l Nb(V) or 2.5×10^{-3}—4.5×10^{-3} mol/l Fe(III). It was found that the yields of complex formation in both NOR–Nb-SCN and NOR–Fe-SCN systems under the optimum formation conditions were 100%.

The optimum formation conditions of ion association complexes in NOR–Nb-SCN and NOR–Fe-SCN systems are

Table 1. Optimum Formation Conditions of the Ion Association Complexes in NOR–Nb-SCN and NOR–Fe-SCN System

Conditions	Ion association complex in system	
	NOR–Nb-SCN	NOR–Fe-SCN
Reaction time (min)	5	5
Time of extraction (min)	2	2
Temperature (°C)	25	25
Concentration of acid (mol/l)	2—3 ($HClO_4$)	1.2—1.8 (H_2SO_4)
Concentration of thiocyanate (mol/l)	1.8—2.5	0.9—1.2
Concentration of niobium(V) or iron(III) (mol/l)	1.2×10^{-3}—2.5×10^{-3}	2.5×10^{-3}—4.5×10^{-3}
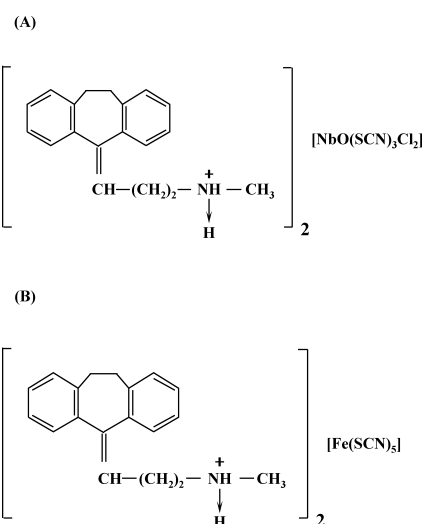
Fig. 2. Suggested Structures of Ion Association Complexes (NOR H)$^+$[NbO(SCN)$_3$Cl$_2$]$^{2-}$ (A) and (NOR H)$^+$[Fe(SCN)$_5$]$^{2-}$ (B)

shown in Table 1.

The composition of ion-pairs was determined by Job's method using equimolar solution. Job's graphs show that the ratio of Nb : NOR or Fe : NOR is 1 : 2 in both associates. Also, the mole-ratio method showed the formation of 1 : 2 ion-pairs. The obtained results indicate that 1 : 2 [Nb(V) or Fe(III)] : [drug] ion association complexes are formed through the electrostatic attraction between a positive protonated drug, NORH$^+$, and thiocyanate negative complexes [NbO(SCN)$_3$Cl$_2$]$^{2-}$ or [Fe(SCN)$_5$]$^{2-}$ as shown by the proposed structures (A) and (B), respectively. The structure of the ion association complexes is given in Fig. 2.

Linearity and Range Beer's law range, molar absorptivity, regression equation and correlation coefficient determined for each method are given in Table 2. A linear relationship was found between the absorbance at λ_{max} and the concentration of the drug in the range 15—100 μg/ml and 5—24 μg/ml using the Nb-SCN and Fe-SCN methods, respectively. Regression analysis of Beer's plots at λ_{max} reveals a good correlation. The graphs show negligible intercept and are described by the regression equation obtained by the least-squares method. The correlation coefficients were between 0.9995—0.9996 indicating good linearity. The high molar absorptivities (8.70×10^3 and 1.19×10^4 l mol^{-1} cm^{-1}) of the resulting coloured complexes indicate the high sensitivity of the methods.

Validation of the Methods Examined samples were prepared and tested at four levels of drug using the proposed procedures. The complete set of validation assays was performed for the drug as determined by the proposed methods. The results obtained for the pure drug are given in Table 2. The precision and accuracy of the methods were tested by analyzing six replicates of the drug. The standard deviation, relative standard deviation, recovery and 95% confidence limits of different amounts tested were determined from the calibration curve, as recorded in Table 2. The accuracy of the methods is indicated by the excellent recovery (100.34—99.32)% and (99.67—100.28)% for Nb-SCN and Fe-SCN, respectively, and the precision is supported by the low standard deviation <0.32.

Precision, Accuracy and Specificity Day-to-day precision and accuracy were evaluated by analyzing six samples of three different concentrations, which were prepared and

analyzed on the same day (Table 3). Sample-to-sample variability was assessed using six samples of three different concentrations analyzed on four different days over a period of a week. These results show the accuracy and reproducibility of the assay. Thus, it was concluded that there were no significant intra-day or inter-day differences for the assay. The validity of the proposed methods was also assessed by applying the official method.[2,3] The data indicate that the methods have good accuracy and precision.

The performance of the proposed methods was compared with other existing UV–visible spectrophotometric methods (Table 4). It is evident that the proposed methods are more sensitive then the majority of others reported, due to their higher molar absorptivities and better accuracy. Only the method in reference 8 is more sensitive than recommended methods, but it exhibits disadvantages in terms of difficult reaction conditions necessary for nortriptyline determination. In this procedure maximum absorption was reached at 80 °C heating for 10 min for colour intensity development.

In conclusion, the proposed methods are found to be simple and can compete with other existing spectrophotometric methods in determining presence of the drug at lower concentrations. Results expressed as relative standard deviation (RSD) less than 0.5% is considered very satisfactory.

Interferences In pharmaceutical analysis it is important to test the selectivity towards the excipients and fillers added to the pharmaceutical formulations. The effect of the presence of several species which can occur in real samples with nortriptyline were investigated. The influence of foreign compounds commonly accompanying NOR in pharmaceutical preparations was investigated by preparing solutions containing 10 μg/ml of the drug and increasing concentrations of potential interferent. The level of interference was considered acceptable if the error was no greater than 2% in the analyti-

Table 2. Analytical Characteristics, Precision and Accuracy of the Methods

Parameters	Proposed methods	
	Nb-SCN	Fe-SCN
λ_{max} (nm)	360	490
Beer's law (μg/ml)	15—100	5—24
Molar absorptivity ε (l mol^{-1} cm^{-1})	8.7×10^3	1.19×10^4
Linear regression equation, $Y^{a)}$		
Slope (a)	0.0087	0.034
Intercept (b)	0.012	0.005
Limit of detection, LD (μg/ml)	0.84	0.32
Limit of quantification, LQ (μg/ml)	3.52	0.98
Correlation coefficient, R	0.9995	0.9996
RSD (%, $n=6$)	0.24	0.32

a) $Y=ax+b$, where x is the concentration in μg/ml.

Table 3. The Inter- and Intra-day Precision and Accuracy Data for Nortriptyline Hydrochloride Determination Obtained by the Proposed Methods, $n=6$

Method	Added NOR (μg/ml)	Inter-day			Intra-day		
		Found (μg/ml)	Precision RSD (%)	Accuracy Er (%)	Found (μg/ml)	Precision RSD (%)	Accuracy Er (%)
NOR–Nb-SCN	20.00	20.02	0.68	0.10	19.94	0.48	−0.30
	30.00	29.92	0.42	−0.26	30.14	0.32	0.46
	60.00	60.16	0.24	−0.26	59.92	0.24	−0.13
NOR–Fe-SCN	8.00	7.98	0.58	−0.25	7.96	0.42	−0.50
	12.00	12.08	0.42	0.66	11.96	0.34	−0.33
	20.00	19.89	0.18	−0.55	20.14	0.24	0.70

Table 4. Comparison of the Proposed Methods with Existing Spectrophotometric Methods for the Assay of Nortriptyline in Pharmaceutical Preparations

Reagents	λ_{max} (nm)	Beer's law (μg/ml)	Molar absorptivity (l mol^{-1} cm^{-})	Recovery (%)	RSD (%)	References
7,7,8,8-tetracyanoquinodimethane	567	1—10	3.33×10^4	100.14±0.89	—	[8]
Quinhydrone	497	12—120	2.94×10^3	99.55—100.1	0.15	[26]
p-Chloranil	560	15—180	1.59×10^3	99.59—100.15	0.55	[26]
p-Chloranil with acetaldehyde	650	5—50	6.32×10^3	99.68—100.07	0.59	[26]
3-Methylbenzothiazolin-2-one hydrazone	620	0.6—30	3.7×10^3	100.2—100.3	0.035	[27]
Nb-SCN	360	15—100	8.7×10^3	99.8—100.2	0.24	This work
Fe-SCN	490	5—24	1.19×10^4	99.7—100.3	0.32	This work

cal signal of NOR. The obtained results for different interfering compounds were as follows (concentration in μg/ml): magnesium stearate, 600; silicon dioxide, 100; starch, 200; glucose, 800; sodium lauryl sulphate, 400; sodium saccharin, 1000; glycerin, 600; talc, 200. The proposed method was successfully applied to the determination of NOR hydrochloride in pharmaceutical preparations.

Determination of Nortriptyline in the Presence of Its Degradation Product The selectivity of the proposed procedures was checked by analysis of different samples of intact drug in the presence of varying amounts of its degradation product, dibenzosuberone. The different synthetic mixtures of nortriptyline and dibenzosuberone were prepared at various percentage concentrations of the degradation product ranging from 10 to 70% calculated with respect to the total weight of sample. The concentration of nortriptyline in the prepared mixtures was determined by the proposed extractive spectrophotometric methods and compared with those obtained by official methods.[2,3] Satisfactory results are presented in Table 5, indicating the high accuracy of the proposed methods. The mean percentage recoveries found were (99.63±0.42)% and (99.46±0.28)% for nortriptyline hydrochloride using niobium(V) and iron(III) thiocyanate methods, respectively. They indicate that the methods are not affected by the presence of up to 70% of the degradation product of nortriptyline, dibenzosuberone as shown in Table 5. The methods can be used as an indication of stability for nortriptyline hydrochloride determination.

The homogeneity of the produced degradation product of nortriptyline, dibenzosuberone, has been studied by TLC on silica gel GF$_{254}$. TLC plates (Merck) were developed with a mobile phase of benzene : dioxane : ammonia 25% (1 : 8 : 1,v/v/v). The obtained results of separation of nortriptyline and its degradation product were good; *Rf* values of the intact drug and its dibenzosuberone were observed at 0.68 and 0.80, respectively.

Formation of the degradation product of nortriptyline was confirmed by the respective UV and IR spectra.

The UV absorption spectrum of the degradation product of nortriptyline, dibenzosuberone, is recorded in the region 200—350 nm. Absorption spectrum of the intact drug shows a maximum absorption band at 230 nm. The UV spectrum of the degradation product exhibits the maximum absorption band at 264 nm, which disappears in the UV spectrum of nortriptyline, suggesting that the produced product is dibenzosuberone.[9]

The IR spectra of the degradation product dibenzosuberone, and intact nortriptyline were measured in the region 400—4000 cm^{-1} and showed several differences, of which the most significant was the appearance of a band at 1640 cm^{-1} in the spectrum of the degradation product. This band confirms the presence of the C=O group. The disappearance of a wide band at 2400—2700 cm^{-1} in the spectrum of the degradation product of nortriptyline confirms that the methylaminopropylidene group in NOR hydrochloride is removed during the formation of dibenzosuberone. These mentioned observations suggest that the degradation product of nortriptyline is dibenzosuberone.

Solid Phase Extraction (SPE) of NOR The proposed method was applied to the determination of NOR after its SPE isolation from tablets. The solid phase extraction (SPE) procedure allowed the selective isolation of NOR from the complex matrix by absorption onto an appropriate sorbent. The interfering impurities were removed by washing with a suitable solvent system and the selective recovery of the retained analyte was obtained by a modified solvent system of suitable elution strength. The process can be modified depending on selection of the sorbent and solvent system, so that interfering components are retained by the sorbent and the analyte is recovered in the filtrate eluate. In order to select appropriate solvents to elute interferences from C$_{18}$ columns, different solvents were checked, *e.g.* acetone, water, acetonitrile, chloromethane, acetate buffer and mixtures of these solvents. Correct selection of the washing solvent was the most important for obtaining good recoveries of the studied drug by the SPE procedure. The best results were obtained using 0.5 ml of a chloroform–acetone mixture (1 : 1, v/v) for instance.

A new SPE procedure for isolation of a studied drug from tablets was used. The preparation procedures of samples usually include a deproteinisation step with organic solvents (methanol or ethanol) or inorganic salts. It was observed that the deproteinisation operation has been the source of serious error, probably due to coprecipitation and adsorption of studied compounds on protein precipitate. The introduction of this step into elaborated SPE procedures resulted in very poor recoveries (about 25%), therefore, this operation was abandoned. Use of the chloroform–acetone mixture (1 : 1, v/v) and organic solvents (dichloromethane and acetonitrile) to elution of interferences has effectively removed almost all excipients of tablets and for this reason, the sorbent materials were not plugged. This allowed the SPE column to be used a long time.

The aim of this study was simplification of the SPE procedure by elimination of the evaporation step of eluent. For this purpose different volumes of organic solvents (chloroform–*n*-butanol) used for the spectrophotometric determination of NOR were checked. The optimal volume of solvent found depended on the type of sorbent material and kind of drug examined. For elution of NOR, a 6 ml mixture of chloroform–*n*-butanol was used (2 : 1, v/v). In every instance, the selected elution solvents provided the cleanest samples which could be directly analyzed by the proposed extractive spectrophotometric methods. The accuracy of the proposed methods was determined by analyzing commercial samples spiked with a known quantity of the drug. Recoveries of the

Table 5. Determination of Nortriptyline Hydrochloride in Presence of Its Degradation Product—Dibenzosuberone in Laboratory Prepared Mixtures by the Proposed Extractive Spectrophotometric Methods

Sample No.	Degradation product of nortriptyline (%)[a]	The proposed methods Intact nortriptyline recovery (%)[b]		The official BP method Intact nortriptyline recovery (%)
		A	B	
1	10	100.06	98.96	99.82
2	20	99.87	99.64	100.08
3	40	98.86	100.24	98.98
4	50	99.89	98.92	99.84
5	60	98.92	100.02	100.04
6	70	100.16	98.96	99.74

a) Calculated with respect to the total weight (mixture of drug-degradation product). *b*) Found pure sample.

drug from the C_{18} column were surprisingly high, uniform and stable in the range (98—102)%. The abovementioned values of recoveries were obtained for samples investigated directly within 24 h. Some results are given in Table 6. The precision of the method indicated by the RSD was good. Connection of the use the SPE procedure of sample preparations with spectrophotometric methods for determination of the drug resulted in high analyte recovery, reduced time for analysis and use of toxic organic solvents and made multistep procedures (evaporation, deprotonisation) unnecessary.

Recovery Studies When the commercial pharmaceutical samples were subjected to the SPE procedures prior to spectrophotometric assay, interference was eliminated and accurate analyses were made. The SPE method provided an effective clean-up of pharmaceutical sample solutions. Recovery studies of the compounds from pharmaceutical preparations were carried out by spiking 1 ml aliquots of commercial samples in triplicate with the pharmaceuticals at three different concentration levels (8, 20, 30 μg/ml). Spiked samples were stored at room temperature for 24 h and the sample extraction described in the sample preparation section was applied. Signals obtained from spiked samples were compared with signals from samples without standard addition and with those obtained by injecting standard solutions.

Recoveries obtained from samples before SPE and after SPE isolation at concentration levels of 8, 20 and 30 μg/ml are given in Table 7. The recoveries of pharmaceuticals were in the range from 93.80 to 102.85% before SPE and in the range of 99.79—100.70% after SPE isolation. RSD of the determination of the selected compounds from pharmaceutical preparations ranged from 0.24 to 0.92%

Application to a Commercial Pharmaceutical Preparation The methods were applied to the determination of NOR in commercial formulations. Their applicability for the assay of nortriptyline in formulations was examined by analyzing various formulations and the results are tabulated in Table 8. Five replicate determinations were made; satisfactory results were obtained for the drug and were in a good agreement with the label claims (Table 8). The results were reproducible with low RSD values. The obtained results of analysis of the commercial formulations and study of the recovery of the drug suggested that there was no interference from any excipients (such as starch, silicon dioxide, magnesium stearate, glucose, glycerin, talc, sodium lauryl sulphate or sodium saccharin) which are offered in tablets. These results were statistically compared with those obtained by HPLC[25] and the official method[2] and there was no significant difference between them. The good agreement of the results indicates the suitability of these extractive spectrophotometric methods for the determination of nortriptyline hydrochloride. In addition, use of these procedures involves considerable savings in cost and time.

Table 6. Results of the Extractive Spectrophotometric Determination of NOR after SPE Isolation from Tablets (*Aventyl*, Leciva)

Drug	Concentration added (μg/ml)	Recovery (%)	RSD (%)
NOR[a]	50.07	100.12	0.48
	50.13	99.94	
	49.89	98.96	
	48.98	100.21	
	50.12	101.16	
	49.78	99.86	
NOR[b]	10.06	99.84	0.69
	9.89	98.96	
	9.96	100.32	
	10.02	100.14	
	9.84	99.68	
	10.08	98.97	

a) By Nb(V)-SCN method; *b*) by Fe(III)-SCN method.

Table 7. Assay Results for the Extractive Spectrophotometric Analysis of Pharmaceutical Compounds before and after SPE Isolation from Tablets

Drug	Before SPE			After SPE		
	Mean found (μg/ml)	Recovery (%)	RSD (%)	Mean found (μg/ml)	Recovery (%)	RSD (%)
NOR[b] (8 μg/ml)	8.11	101.38	0.52	8.02	100.25	0.24
NOR[a,b]	20.57[a]	102.85	0.92	20.14	100.70	0.39
(20 μg/ml)	18.76[b]	93.80	0.82	19.96	99.79	0.58
NOR[a] (30 μg/ml)	28.75	95.83	0.74	29.96	99.86	0.46

a) By Nb-SCN method, *b*) by Fe-SCN method.

Table 8. Results of Determination of Studied Active Substance in Commercial Formulations

Determination substance	Pharmaceutical formulation	Labelled amount (mg)	Determination amount				Error[b] (%)	
			By described method[a]		By EP[2] (mg) (*n*=6)	By HPLC method[25] (mg) (*n*=6)	A	B
			A (mg) (*n*=6)	B				
Nortriptyline hydrochloride	Tablets, Aventyl (Leciva, Czech Republic)	25	25.04	24.92	25.06	24.92	+0.16	−0.31
Nortriptyline hydrochloride	Capsules, Aventyl (Leciva, Czech Republic)	50	50.10	49.92	50.06	50.02	+0.20	−0.16
Nortriptyline hydrochloride	Capsules, Aventyl (Leciva, Czech Republic)	75	74.89	75.24	75.20	74.92	−0.15	+0.32

a) Described extraction spectrophotometric methods (A–Nb-SCN, B–Fe-SCN). *b*) Error (%) *vs.* labeled amount.

Conclusion

A rapid, simple, sensitive and accurate extractive spectrophotometric methods have been developed which can be used for determination of NOR hydrochloride in pharmaceutical formulations. There are some methods[6,7,11,21,26,27] available for this determination which use UV–visible spectrophotometry; however, these have several disadvantages: the cost of equipment, manner of performance, time required, difficulty of reaction conditions and analytical procedures.

We described the extractive spectrophotometric methods for determination of NOR based on the formation of ion pair complexes with thiocyanate of niobium(V) and iron(III). The methods make use of simple reagents, which an ordinary analytical laboratory can afford, and validation showed them to be suitable for routine determination of NOR in its formulations. The commonly used additives such as starch, silicon dioxide, magnesium stearate, glucose, glycerin, talc, sodium lauryl sulphate and sodium saccharin do not interfere with the assay procedures. The procedures could be used to determine the drug in the presence of its introduced degradation product, dibenzosuberone. The proposed extractive spectrophotometric methods can also be used for the determination after its isolation from tablets by solid phase extraction (SPE). The main advantages of these procedures are low cost of reagents and apparatus used and short time of analysis. Both methods are characterized by good precision, reproducibility of determination and high sensitivity.

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