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Stability indicating method for determination of nortriptyline hydrochloride using 3-methyl-2-benzothiazolinone hydrazone (MBTH)

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Abstract

A spectrophotometric procedure is described for determination of nortriptyline hydrochloride in pure and dosage form as well as in the presence of its degradate. 3-Methyl-2-benzothiazolinone hydrazone (MBTH) has been used as the chromogenic reagent, where aqueous solutions of the drug and reagent are treated with cerium(IV) ammonium sulphate in an acidic medium. Nortriptyline hydrochloride reacts to give a blue coloured product having two absorption maxima at 619 and 655 nm. Various parameters affecting the reaction have been studied. Beer's law is obeyed in the concentration range of 24–216 μg ml^{-1} of nortriptyline hydrochloride, with mean percentage recoveries of 100.22 ± 0.870 and $100.66 \pm 0.642\%$ for both maxima, 619 and 655 nm, respectively. Results were statistically analyzed and compared with those obtained by applying the British Pharmacopoeia (1993) method.

Keywords: Spectrophotometry; Stability indicating method; Determination of nortriptyline hydrochloride; 3-Methyl-2-benzothiazolinone hydrazone reagent (MBTH)

1. Introduction

Nortriptyline hydrochloride (Allegron; Noritren; Nortrilen; Pamelor) is an ingredient of Motipress and Motival tablets. Its formula is 3-(10,11-Dihydro-5H-dibenzo[*a*,*d*]

cyclohepten-5-ylidene)-*N*-methyl propylamine hydrochloride, and it is used as an antidepressant [1].

Various analytical procedures have been reported for determination of this drug. They include spectrophotometry [2,3], capillary electrophoresis [4], infrared immunoassay [5], radioimmunoassay [6,7], thin layer chromatography [8,9], gas chromatography [10–12] and high pressure liquid chromatography [13,14].

Nortriptyline hydrochloride is determined by the non-aqueous titration method as reported in British Pharmacopoeia (1993) [15], US Pharmacopoeia (1995) [16] and the European Pharmacopoeia (1997) [17].

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3-Methyl-2-benzothiazolinone hydrazone (MBTH) in the presence of oxidizing agents has been used as a sensitive chromogenic reagent for spectrophotometric determination of some nitrogen-containing compounds. These include ranitidine HCl [18], metoclopramide [19], sulpha drugs [20], some diuretics [21], local anaesthetics [22], cholesterol [23], carazolol and pindolol [24], phenolic drugs [25,26], aldehydes [27], benzodiazepines [28], sympathomimetic agents [29] and some phenothiazine derivatives [30].

The aim of this work is to develop a simple, accurate and reasonably selective method for the determination of nortriptyline HCl in the presence of its degradate which is pharmacologically inactive by using 3-methyl-2-benzothiazolinone hydrazone (MBTH). The proposed method was successfully applied for the determination of nortriptyline HCl in bulk powder and in its pharmaceutical dosage form.

2. Experimental

2.1. Apparatus

A UV-1601 PC-UV visible Shimadzu spectrophotometer was used.

2.2. Samples

2.2.1. Nortriptyline hydrochloride

This was obtained from Bristol-Myers Squibb Egypt. It was assayed by the British Pharmacopoeia (1993) method [15] and was found to contain $99.92 \pm 0.584\%$.

2.2.2. Motival tablets

These were produced by Bristol-Myers Squibb Egypt and were purchased from local pharmacies. Each tablet was labelled to contain 10 mg nortriptyline HCl and 0.5 mg fluphenazine HCl. The Batch No. was A 80121.

2.2.3. Degraded nortriptyline HCl

This was prepared by adding 1 ml of 30% hydrogen peroxide to 0.3 g of drug in 50 ml distilled water and then heating to drive off excess oxygen, followed by cooling. The course of the reaction was followed by UV–Visible spectrophotometry (200–400 nm) and by TLC using silica gel 60 F_{254} TLC plates and a mobile phase of benzene/methanol/ammonia (9:1:0.1 v/v/v). The induced degradate was confirmed by IR spectrometry and was also tested with saturated sodium bisulphite which resulted in the formation of a white precipitate confirming the presence of the (C=O) group.

2.3. Stock solutions

2.3.1. Nortriptyline hydrochloride standard solution

This solution (0.6 mg ml^{-1}) was prepared by dissolving 60 mg of nortriptyline HCl in distilled water in a 100-ml volumetric flask.

2.3.2. Preparation of nortriptyline degradate

Ten milliliters of the previously prepared (see Section 2.2.3) degraded nortriptyline (equivalent to 60 mg) was transferred into a 100-ml volumetric flask and diluted to volume with distilled water.

2.3.3. Laboratory prepared mixtures

Mixtures containing different ratios of nortriptyline HCl and its laboratory prepared degradate were prepared, to contain 10–80% of the degradate.

2.4. Chemicals and reagents

The following chemical and reagents were used: MBTH solution: 0.2% w/v, freshly prepared in distilled water, obtained from Sigma (USA); Cerium(IV) ammonium sulphate solution, 1% w/v in 5% sulphuric acid; sulphuric acid (E. Merck, W. Germany); hydrogen peroxide 30% w/v; Adwic; ethanol, methanol, acetone, acetonitrile, isopropyl alcohol, benzene and dimethylsulphoxide were obtained from E. Merck, Germany; Silica gel 60 F_{254} TLC plates were also from Merck.

3. Procedure

3.1. Construction of calibration graph

About 60 mg of nortriptyline hydrochloride was accurately weighed, dissolved in distilled water and diluted to volume in a 100-ml volumetric flask. Different portions of this standard solution (1–9 ml), were transferred into a series of 25-ml volumetric flasks. Complementary volumes of distilled water were added to adjust the volume to 9 ml then 2 ml of MBTH solution (0.2% w/v) and 2 ml of cerium(IV) ammonium sulphate (1% w/v). The contents were mixed thoroughly and after 1 h, made up to volume with distilled water. The absorbances of the solutions were measured at 619 and 655 nm against a blank similarly prepared.

3.2. Application to pharmaceutical formulation

3.2.1. Motival tablets

The coloured coats of 30 tablets were removed with water, air dried, powdered and mixed well, then a quantity of the powdered tablets equivalent to 30 mg of nortriptyline hydrochloride was accurately weighed into a 50-ml volumetric flask. Distilled water was added and made up to the mark. The flask was then shaken for 15 min using an ultrasonic shaker or magnetic stirrer. The solution was then filtered into a dry conical flask using dry filter paper and a dry funnel. The filtrate was finished as detailed under Section 3.1 for a standard solution.

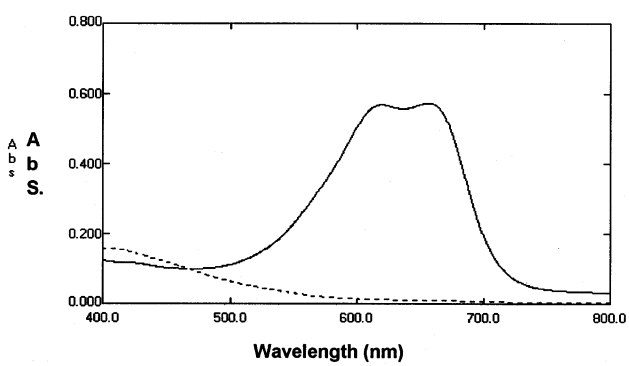

Fig. 1. Absorption spectra of nortriptyline HCl–MBTH reaction product (120 μg ml^{-1}, solid line) and reagent blank (dashed line).

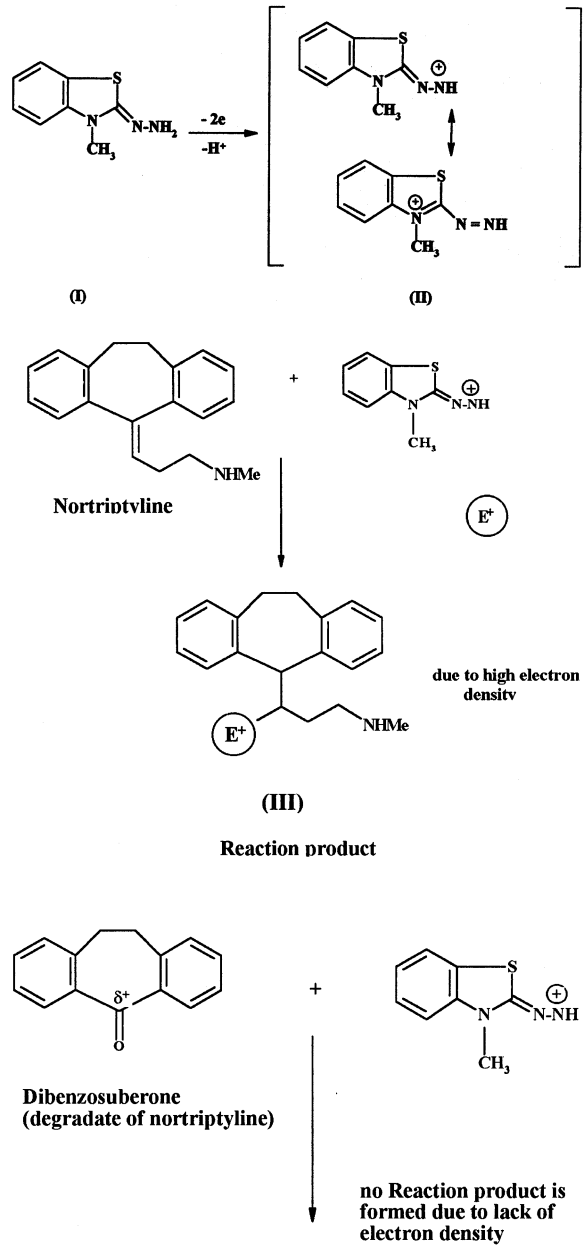

Fig. 2. Mechanism of the reaction of MBTH with nortriptyline HCl and its degradate.

3.2.2. Stability study

A degraded sample of nortriptyline hydrochloride solution was prepared as mentioned under Section 2.2.3. An accurate portion equivalent to 60 mg of nortriptyline degraded solution was transferred into a 100-ml volumetric flask and diluted to volume with distilled water. The standard drug solution was mixed with its degraded sample in different ratios, within the concentration range 24–216 μg ml^{-1}. The solution was finished as mentioned under Section 3.1. The procedure was adopted to prepared mixtures of the authentic samples of nortriptyline HCl and its laboratory prepared degradate. Absorbances of these laboratory prepared mixtures were measured at 619 and 655 nm.

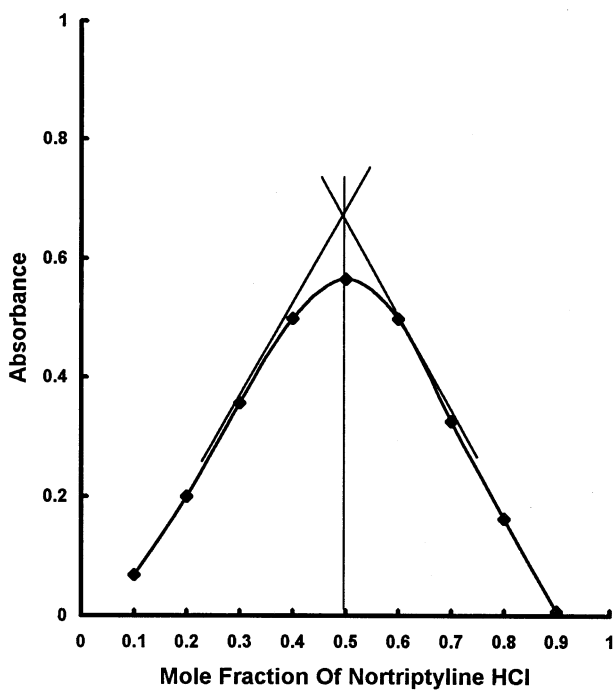

Fig. 3. Stoichiometry of the reaction of nortriptyline HCl with MBTH by the continuous variation method using 2×10^{-3} M solutions.

4. Results and discussion

4.1. Reaction involved

The absorption spectrum of the reaction product of nortriptyline HCl with MBTH in the presence of cerium(IV) ions is shown in Fig. 1. The reaction of MBTH with nortripyline HCl in the presence of an oxidant [31], proceeds via oxidative coupling [32]. MBTH (**I**) loses two electrons and one proton on oxidation with oxidizing agent cerium(IV) ammonium sulphate, forming the electrophilic intermediate (**II**), which is the active coupling species [19,33]. The reagent would be expected to attack carbon atom with maximum electron density as in nortriptyline hydrochloride to form the coloured product (**III**), but no coloured product is formed with its degradate, namely dibenzosuberone [34], due to the lack of electron density at the attack site, according to the scheme shown in Fig. 2.

This pathway for the reaction of MBTH with nortriptyline HCl is also confirmed by the results obtained upon applying the continuous molar variation method [35]. This investigation showed that nortriptyline HCl interacts with MBTH in the ratio 1:1 when using 2×10^{-3} M of both drug and MBTH as shown in Fig. 3.

4.2. Effect of reaction variables

Five variables were found to affect the intensity of the resulting colour: reagent concentration, cerium(IV) ammonium sulphate concentration, acid concentration, development time and type of diluting solvent.

4.3. Effect of MBTH concentration

When various concentrations of MBTH solutions (0.1–0.4% w/v) were added to a fixed concentration of the drug, 2 ml of 0.2% solution was found to be sufficient to produce maximum colour intensity.

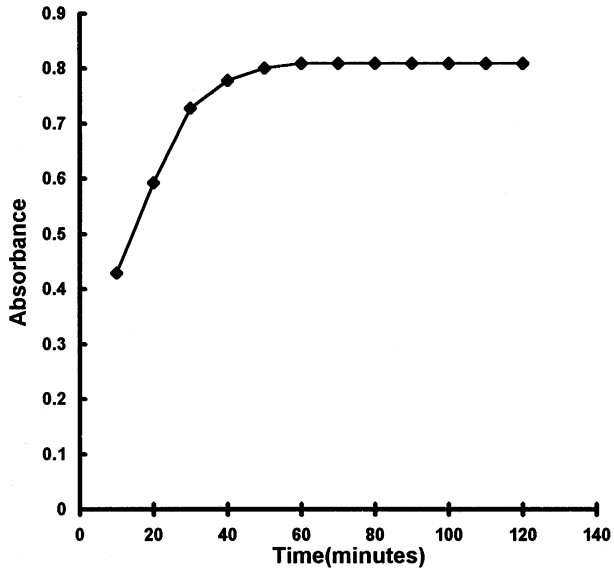

Fig. 4. Effect of reaction time on the absorbance of the reaction product formed (168 μg ml^{-1}).

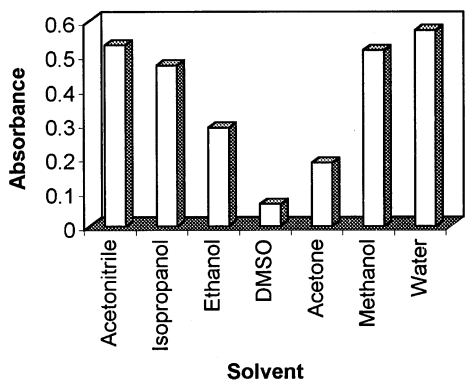

Fig. 5. Effect of diluting solvent on the reaction between nortriptyline HCl (120 μg ml^{-1}) and MBTH.

4.4. Effect of cerium(IV) concentration

The optimum concentration of cerium(IV) ammonium sulphate solution leading to maximum colour stability was found to be 2 ml of 1% solution. Higher concentrations gave colours which faded rapidly with time, probably owing to the formation of several oxidation products or the oxidation of the chromogen itself [29].

4.5. Effect of sulphuric acid concentration

The optimum concentration of H_2SO_4 in which cerium(IV) ammonium sulphate was dissolved was found to be 5%. Higher concentrations did not affect the colour intensity.

4.6. Effect of development time

The proposed procedure was applied as detailed under Section 3.1, by keeping the solutions for different time intervals (10–120 min). The maximum colour intensity was obtained after 1 h. The colour was stable for a further 1 h as shown in Fig. 4.

4.7. Effect of the diluting solvent

The solvents used to carry out the reaction with MBTH were studied using water, ethanol, methanol, isopropyl alcohol, acetone, acetonitrile and dimethylsulphoxide. Referring to Fig. 5, it is obvious that both water and acetonitrile give highest absorbances. Statistical comparison showed that there is no significant difference upon using the two mentioned solvents. Water was used throughout the work on the grounds of cost and availability.

4.8. Quantification

A linear correlation was obtained between absorbance at both λ_{max} and concentration of the drug. Linearity was achieved in a concentration range of 24–216 μg ml^{-1} as given in Table 1. Correlation coefficients, molar absorptivities, detection limits, range intercepts, slopes and regression equations for the calibration data of the drug are given in Table 1. Linear Beer's law graphs for the investigated drug can also be used for the calculation of concentration. The minimum detectable amount was found to be 12 μg ml^{-1}. Mean percentage accuracies were found to be 100.22 ± 0.870 and 100.66 ± 0.642% at 619 and 655 nm, respectively as shown in Table 2.

Reproducibility of results obtained by analysis of the same samples under a variety of test conditions which define the ruggedness of an analytical

Table 1
Spectral characteristics of the reaction product of MBTH with nortriptyline hydrochloride[a]

Parameter[b]	Nortriptyline HCl with MBTH	
	619 nm	655 nm
Linear range (μg ml^{-1})	24–216	24–216
Limit of Detection (LOD) (μg ml^{-1}) [16]	12	12
Limit of Quantitation (LOQ) (μg ml^{-1})	24	24
Accuracy	100.22 ± 0.870	100.66 ± 0.642
Precision	0.873	0.644
Intercept (a)	0.0118	0.0135
Slope (b)	0.0047	0.0047
Correlation coefficient (r)	0.9998	0.9999
Mean	100.22	100.66
S.D.	0.872	0.646
R.S.D.	0.870	0.642
ε (apparent molar absorptivity)	1.5×10^3	1.5×10^3
Regression equation	$A = 0.0047C + 0.0118$	$A = 0.0047C + 0.0135$

[a] A, absorbance; C, concentration (μg ml^{-1}) of nortriptyline HCl.
[b] These results are the average of at least five experiments.

method was determined and satisfactory results were obtained.

The validity of the derived regression equations was assessed by the determination of the drug in its dosage form (Motival tablets). Results obtained by applying the standard addition technique are shown in Table 3. Mean percentage recoveries of added nortripyline HCl to tablet were found to be 99.72 ± 0.688 and 100.06 ± 0.771% using λ_{max} of 619 and 655 nm, respectively. This shows that tablet additives including dibasic calcium phosphate, corn starch, gelatin, magnesium stearate, talc, lactose and other active constituents namely fluphenazine HCl incorporated with nortriptyline HCl in Motival tablets did not interfere.

Degradation of nortriptyline HCl was studied using hydrogen peroxide as an oxidant [36]. The degradation product was expected to be dibenzosuberone [34], a pharmacologically inactive agent [37]. Its structure was confirmed by IR spectrometry where (C=O) at 1640 cm^{-1} was assigned. It was also tested with sodium bisulphite which resulted in formation of a white precipitate adduct confirming the presence of the (C=O) group [38]. It was also reported to be one of the impurities of

Table 2
Spectrophotometric determination of authentic nortriptyline HCl using MBTH (at 619 and 655 nm)

The proposed procedure with MBTH					Official method B.P. (1993) [15]
Taken (μg ml^{-1})	619 nm		655 nm		
	Found[a] (μg ml^{-1})	Recovery (%)	Found[a] (μg ml^{-1})	Recovery (%)	
24.00	23.83	99.29	24.21	100.89	
48.00	48.72	101.51	48.68	101.42	
72.00	72.13	100.18	72.51	100.71	
96.00	95.96	99.96	96.55	100.58	
120.00	118.30	98.58	118.89	99.08	
144.00	144.89	100.62	145.28	100.89	
168.00	168.72	100.43	169.11	100.66	
192.00	193.83	100.95	194.00	101.04	
216.00	217.00	100.47	217.40	100.65	
Mean ± R.S.D.		100.22 ± 0.870		100.66 ± 0.642	99.92 ± 0.584

[a] Average of five experiments.

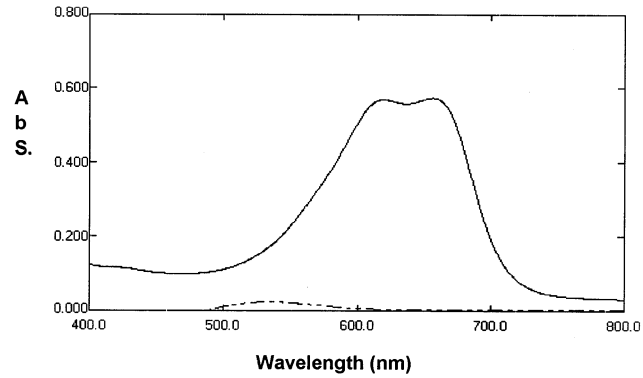

Fig. 6. Absorption spectra of the products of the reaction between MBTH and nortriptyline HCl (120 μg ml^{-1}, solid line) and its degradate (dashed line).

Table 3
Standard addition technique for the spectrophotometric determination of nortriptyline HCl via reaction with MBTH in Motival tablets

Taken (μg ml^{-1})	Authentic added (μg ml^{-1})	Motival tablets			
		Authentic found[a] (μg ml^{-1})		Recovery (%)	
		619 nm	655 nm	619 nm	655 nm
48.00	–	–	–	99.81	99.06
48.00	24.00	23.83	24.05	99.29	100.21
48.00	48.00	47.45	47.88	98.85	99.75
48.00	72.00	71.71	71.07	99.60	98.71
48.00	96.00	95.54	96.17	99.52	100.18
48.00	120.00	120.86	121.07	100.72	100.89
48.00	144.00	144.47	144.90	100.33	100.63
Mean ± R.S.D.				99.72 ± 0.688	100.06 ± 0.771

[a] Average of three determinations.

the drug [34]. The intact drug was found to be 0% after degradation as examined by TLC.

The proposed procedure was used to determine the intact drug in the presence of its degradate. The degradation product does not react with the reagent thus having zero absorbance at the λ_{max} used, as shown in Fig. 6. Table 4 shows the results obtained upon analysis of synthetic mixtures of intact drug and its degradate in different ratios. It is obvious that the proposed procedure can be successfully used for selective determination of the intact nortriptyline HCl in the presence of its degradate up to about 80%.

Results of the proposed procedure were statistically compared with those obtained by adopting the official British Pharmacopoeia (1993) non-aqueous titration method [15]. Table 5 shows that the calculated t- and F-values are less than the

Table 4
Spectrophotometric determination of nortriptyline HCl via reaction with MBTH in synthetic mixtures with its degraded samples

Sample no.	Degradation product[a] (%)	Intact nortriptyline recovery (%)	
		619 nm	655 nm
1	10	100.49	100.31
2	20	99.39	99.55
3	30	99.94	100.12
4	40	99.19	99.41
5	50	100.12	100.18
6	60	100.03	99.61
7	70	99.23	99.32
8	80	99.58	100.23
Mean ± R.S.D.		99.75 ± 0.469	99.84 ± 0.407

[a] Percentage of total weight.

Table 5
Statistical analysis of results obtained by spectrophotometric determination of authentic samples of nortriptyline HCl by the proposed procedure as compared to those obtained by the official method[a]

Item	Nortriptyline HCl with MBTH at		Official method B.P. (1993) [15]
	619 nm	655 nm	
Mean	100.22	100.66	99.92
S.D.	0.872	0.646	0.584
R.S.D.	0.870	0.642	0.584
n	9	9	5
Variance	0.760	0.417	0.341
Student's *t*-test	0.609 (2.179)	1.678 (2.179)	
F-value	2.23 (6.04)	1.22 (6.04)	

[a] Figures in parentheses are the corresponding theoretical *t*- and *F*-values ($P = 0.05$).

theoretical ones, indicating no significant differences between the proposed procedure and the official one. In addition, the MBTH method has the advantages of being simple and selective to the drug in the presence of its degradate.

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