# Analytical methodologies for determination of amitriptyline and its metabolite nortriptyline: A review

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**ABSTRACT**

*Amitrityline and its metabolite nortriptiline are tricyclic antidepressant drugs widely used for the treatment of several psychiatric disorders. Several methods have been published for the determination of these two antidepressant drugs in pharmaceuticals, biological materials and environmental samples. In this review some of analytical techniques such as ultraviolet/visible spectrophotometry, fluorimetry, capillary electrophoresis, and chromatographic methods (gaschromatography and high-performance liquid chromatography) were discussed. Although HPLC and capillary electrophoresis methods are extensively employed in spite of that UV/VIS spectrophotometry are still popular because of the inherent simplicity, low cost, and reliability for determination of drugs in pharmaceutical preparations.*

**Keywords***: Antidepressant, Tricyclic, Amitriptyline, Nortriptyline, Analytical Techniques*
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## INTRODUCTION

Tricyclic antidepressants (TCAs) are three ring chemical structures that were widely used in the clinical practicefor the treatment of different types of depression like phobias, insomnia, chronic pain syndromes, panic disorder, eating disorders (e.g., bulimia nervosa), premenstrual dysphoric disorderand anxiety disorders[1–4].These disorders affect to the patients both economically and socially which can eventually leads to suicidal behaviour. Antidepressant (ATD) drugs are frequently prescribed in various combinations, leading to more possible drug–drug interactions, while dosage is largely based on trial-and-error [5, 6]. All the tricyclic antidepressants are pharmacologically and structurally similar. Normally these agents inhibit the reuptake of three important neurotransmitters (Serotonin, norepinephrine and dopamine) in the central nervous system of brain cells [7].

Amitriptyline hydrochloride is a tricyclic antidepressant and is chemically known as 3-(10, 11-dihydro-5H-dibenzo [a,d] cycloheptene-5-ylidine)-N,N-dimethyl-1-propanamine hydrochloride [8] . It is a white, colourless, crystalline compound which is freely soluble in water. It is used for the treatment of several psychiatric disorders [9 - 11].The usual recommended dose varies between 50 and 200 mg daily. Despite the beneficial effects of amitriptyline hydrochloride the overdoses of the drug had many undesirable side effects and may lead to some disorders like unconsciousness, convulsions, hyperreflexia and cardiac depression [12]. After oral administration, AMI is transformed to its active metabolite (nortriptyline, NOR) by mono-*N*-demethylation(**Figure 1**) and by hydroxylation, leading to the formation of E-10-hydroxy (EHAT) and Z-10-hydroxyamitriptyline (ZHAT). Nortriptyline is further demethylated to desmethylnortriptyline (NNT) and hydroxylated to E-10-hydroxy nortriptyline (EHNT) and Z-10-hydroxynortriptyline (ZHNT). The demethylation of amitriptyline and nortriptyline is mainly catalysed by CYP2C19, with the participation of other CYP enzyme forms in higher drug concentrations. Nortiptyline is chemically known as3-(10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-ylidene)-N-methyl-1-propanamine (NT).

In this review, a wide variety of analytical methods have been reported for the determination of amitriptyline andnortriptyline in pharmaceutical preparations and in biological fluids. These methods include spectrophotometry, high-performance liquid chromatography (HPLC), gas chromatography (GC) and capillary electrophoresis (CE), principally. In this work, we have recompiled the described methods in the literature for determination of these two antidepressants.

## 1. Techniques involved in Pharmaceutical formulations and Biological Fluids

From the literature various analytical methods such as ultraviolet/visible spectrophotometry,fluorimetry, capillary electrophoresisand chromatographic methods (liquid chromatography, gaschromatography and high-performance liquid chromatography)were used for the determination of amitriptyline, and nortriptyline in formulated products and in biological fluids.

### 1.1 Spectrophotometric and Spectrofluorimetric methods

Spectrophotometric method can be regarded as one of the suitable and economical methods of drug analysis, although its importance has not decreased to any extent during modern times. Moreover-mainly as a result of its coupling with other methods-the field of application of spectrophotometry continues to increase. Methods based on the natural ultraviolet-visible absorption of analyte are applied for the determination of active substances in bulk drugs. This approach has certain limitations from the points of the applicability, sensitivity, selectivity and effectiveness of the measurement. Some examples of spectrophotometric applications in quantitative pharmaceutical analysis and biological analysis and are described below.

Mohamed etal. [13] proposed a simple spectrophotometric method based on the formation of charge transfer complex between 7,7,8,8-tetracyanoquinodimethane (TCNQ)and amitriptyline in pharmaceutical formulations. Amitriptyline acts an electron donor and TCNQ as electron accepter. The low value of the relative standard deviation (0.212–0.915) indicate the high accuracy and precision of the method. Also same reagent was used by Oztuncetal.[14] for the determination of nortriptyline which leads to the formation of coloured chromogens in acetonitrile medium at 80°C which shows maximum absorbance at 567 nm. Later Nour El-Dienetal. [15] developed and validated a quantitative spectrophotometric method which involves the    formation of 1,2-dichloroethane extractable yellow colored ion-pair complex between amitriptyline, methyl orange (MO) and bromocresol green (BCG) reagents at 420 and 410nm, respectively.

Mohamedet al. [16] developed a spectrophotometric method based onthe formation of ion-pair between amitriptyline and inorganic complex of Mo(V)–thiocyanate which was extracted withmethylene chloride. N.A. El Ragehyet al. [17]developed and validate a spectrophotometric procedure for determination of nortriptyline hydrochloride in pure form, dosage form as well as in the presence of its degradate. Method was based on the reaction with 3-Methyl-2-benzothiazolinone hydrazone (MBTH) which was used as the chromogenic reagent. The aqueous solution (nortriptyline and MBTH) was then treated with cerium(IV) ammoniumsulphate in an acidic medium to give a blue coloured product having two absorption maxima at 619 and 655 nm. Beer's law was obeyed in the concentration range of 24–216 mg ml$^{-1}$.

Abou Attia [18] described three spectrophotometric methods for the determination of nortriptyline hydrochloride in bulk drugand in tablets. Two methods were based on the charge transfer complex formation between nortriptyline which acts as a n-donor and quinhydrone or p-chloranil which acts as p-acceptor at 497 and 560 nm respectively. Third method was based on the formation of vinylamino substituted quinone by the interaction of *N*-alkylvinylamine formed by the condensation of the free secondary amine group and acetaldehyde with *p*-chloranil. The coloured product exhibits an absorption maximum at 650 nm in dioxane.

Comparison of UV spectrophotometric and LC method was done by Moreno et al. [19] for the determination of nortriptyline hydrochloride in oil: water (o:w) microemulsions and it was found that both methods shows 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.

Revanasiddappa and Manju [20] described oxidative coupling method for the determination of amitriptyline and nortriptyline spectrophotometrically in pure and dosage forms. The reaction was based on coupling of these two drugs with 3-methylbenzothiazolin-2-one hydrazone in the presence of iron(III) chloride prepared in 1 M hydrochloric acid. A Kinetic photometric and fluorometric method  was proposed by La Peiqaetal. [21] for the determination of nortriptyline hydrochloride utilizing 4-chloro-7-nitrobenzofurazan in pharmaceutical preparations and mean recovery was found almost near to 100% with both photometric and fluorimetric detection. A semiautomatic extraction-fluorimetric method for determination of amitriptyline and nortriptyline based on the formation of ion-pair with 9,10-dimethoxyanthracene-2-sulphonate was described by Valenzuela et al.    in

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pharmaceutical formulations. The complex formed was extracted into dichloromethane and complex formation was favoured in strongly acidic medium which showed fluorescence at 448 nm after excitation at 265 nm[22].

The analytical characteristics of the above cited methods and other parameters described in the literature are presented in Table 1.

### 2.2 Capillary electrophoresis

Capillary electrophoresis (CE) is a relatively new analytical technique based on the separation of charged analytes through a small capillary under the influence of an electric field. In this technique solutes are seen as peaks as they pass through the detector and the area of each peak is proportional to their concentration, which allows quantitative determinations. CE separations are generally more efficient, can be performed on a faster time scale, require only nanoliter injection volumes, and in most cases, take place under aqueous conditions. Several reports have appeared on the application of this technique in the routine drug analysis.

Wuet al. [23]developed capillary zone electrophoresis method for the determination of amitriptyline and its active metabolite nortriptyline, in the presence of b-cyclodextrin (0.2 mM). in human plasma. Detection was carried out at 200 nm using clomipramine (1 nmol) as an internal standard. Other parameters were also studied such as the type and concentration of cyclodextrin, pH and concentration of Tris buffer which affects the separation of amitriptyline and Nortriptyline. Chenet al. [24] described a field-amplified sample stacking and capillary electrophoresis method for the determination of amitriptyline and its metabolite nortriptyline in human plasma using Tris buffer (1.4 M, pH 4.50) containing beta-cyclodextrin(beta-CD, 1.0 mM) and 50% (v/v) ethylene glycol at a voltage of 25kV  with a detection wavelength of 200 nm.

Non-aqueous capillary electrophoresis method for simultaneous determination of amitriptyline and nortriptyline was described and validated in pharmaceutical formulations and plasma samples by Cantúet al. [25]. Separation was achieved within 4.3 min at optimized conditions of50mM ammonium acetate, applied voltage of 30 kV, capillary with 48 cm in length. Acceptable precision and linearity were achieved using the internal standard method. Results found were comparable or better than described in the literature for high performance liquid chromatography (HPLC)-based methods.

*N,N,N,N*-tetramethyl-1,3-butanediamine (TMBD)was used as additive in the background electrolyte by  Aquila [26] for the separation of  Amitriptyline and nortriptyline using capillary zone electrophoresis. As these drugs are similar in structure, mass and pka values, therefore their separation, by capillary zone electrophoresis, requires careful attention of parameters, such as the pH and the composition of the electrolyte solution. Kou et al. [27] developed a cyclodextrin-modified capillary zone electrophoresis method for the determination of amitriptyline and nortriptyline in commercial dosage forms. Separation was achieved at a voltage of 20 kV at a wavelength of 200 nm.

### 2.3 Chromatographic Methods

High performance liquid chromatography(HPLC) is the most powerful and versatile instrumental technique used for the detection and quantitation of chemical components in the complex matrices frequently encountered in pharmaceutical analysis[28]. Liquid chromatography combined with mass spectrometry (LC-MS) is considered as one of the most important techniques of the last decade of 20th century [29, 30].Several HPLC and LC-MS methods have been reported for the determination of amitriptyline and nortriptyline biological fluids.

Almudever et al. [31] described a HPLC method for the determination of nortriptyline in rat plasma. Procedure involved the derivatization with 9Hfluoren-9-ylmethyl chloroformate (Fmoc-Cl) and isocratic reversed-phase (C18) chromatography with fluorescence detection. The nortriptyline derivative was formed within 20 minutes and was stable at room temperature for about 48 hours. Later same method (HPLC) coupled with electrospray ionization mass spectrometry was described by Shenet al. [32] for the determination of amitriptyline and nortriptyline in rat plasma using a XB-C4 column (4.6mm×250mm, 5µm, Welch Materials) with a mobile phaseconsisting of 10mM ammonium acetate (0.6‰ formic acid)–acetonitrile (60:40, v/v) at a flow rate of1.0 ml/min. Analysis of amitriptyline and nortriptyline in plasma andhuman liver microsomes was described by Ghahramani and Lennard[33]. Calibration curve was found to be linear over a wide range of concentratio passing through the origin with a detection limit of 2ng/ml.

Tybringet al. [34] described a method using Deuterium labelled internal standards ([ H ]NT and 4 2 [ H ]10-OH-NT) for the quantification of nortriptyline and 10-hydroxy metabolite in plasma. The compounds were separated by reversed-phase HPLC and detected using 3 atmospheric pressure chemical ionisation and mass spectrometry. The method shows good accuracy and precision with limit of quantification of 0.8 ng/mL for both compounds. The

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intraday and interday results show coefficients of variation of 11% at low conc. i.e1.6 ng/ml range, and 7% at 8 ng/mL.
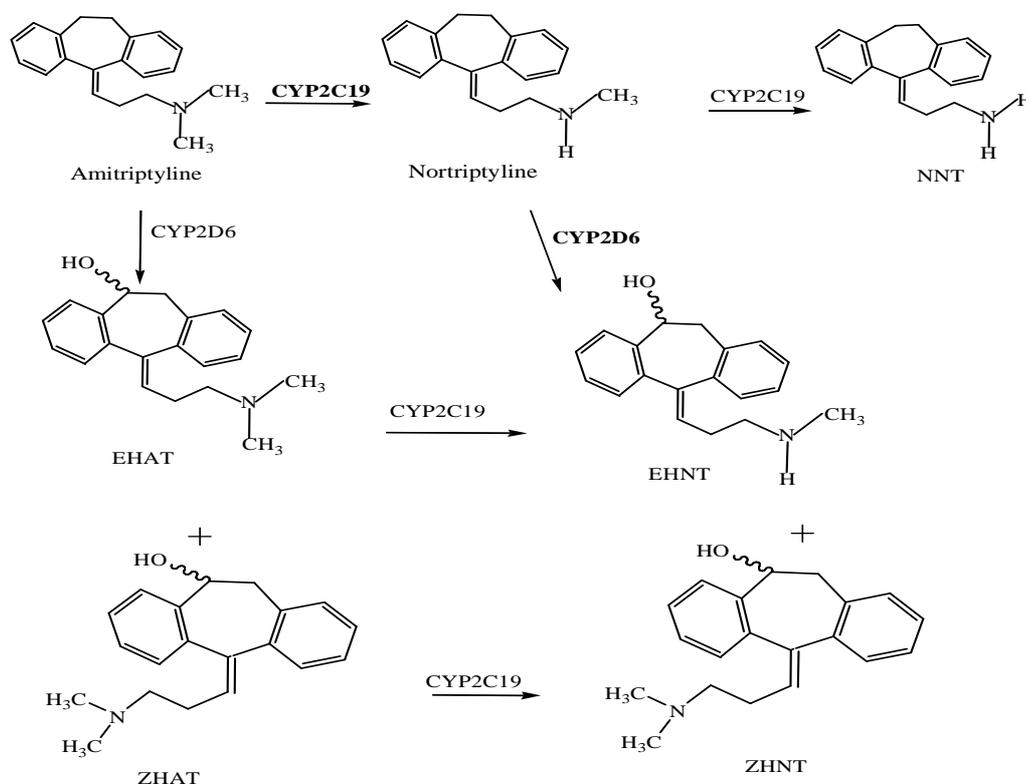


**Figure 1.Main metabolite pathways of amitriptyline**

For the detection of secondary group bearing antidepressant (nortriptyline) Oztuncet al. [35] utilizes 7,7,8,8-tetracyanoquinodimethane (TCNQ) as a new derivatization reagent for HPLC and TLC method in plasma. Displacement reaction between nortriptyline and TCNQ takes place which results in the formation of purple chromogens which were directly separated by either reversed-phase HPLC on a C column 18 using acetonitrile–water (60:40) as mobile phase at 567 nm or TLC on silica gel plates using three developing solvent systems.

Kirchherr and Kuhn-Velten [36] described a HPLC tandem mass spectrometry method for the determination of amitriptyline and nortriptyline in the presence of other antidepressants in human plasma using 5mM acetate buffer on   a monolithic C18 column (50mm×4.6 mm) with methanol gradient at pH 3.9. Set of three internal standards was used with varying hydrophobicity for the quantification of drug. After electrospray ionization positive ion fragments were detected in the multiple reaction monitoring mode with an API4000 tandem mass spectrometer. Good result was found with average correlation coefficient of 0.9988.

Mosavianet al. [37] described a new method for the determination of amitriptyline in waste water by ionic liquid based immersed droplet micro extraction (IL-IDME) prior to high-performance liquid chromatography with ultraviolet detection using 1-Hexyl-3-methylimidazoliumhexafluorophosphate ([C6MIM][PF6]). Various factors that affect extraction, such as volume of ionic liquid, stirring rate, extraction time, Ph of the aqueous solution and salting effect, were optimized. Method was successfully applied to the determination of amitriptyline in the hospital waste water samples.

A fully automated on-line method for the determination of nortriptyline was described by Olesenet al. [38] in human serum using ASPEC XL (Gilson) solid-phase extraction apparatus in combination with high-performance liquid chromatography on cyanopropyl cartridges. HPLC was carried out using a C column with a mobile phase of 18 acetonitrile–0.01 *M* triethylamine (34:66 v/v) buffer using pH 3  and was detected at 242 nm.

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Breaudet al. [39] described a fully-automated turbulent-flow liquid chromatography–tandem mass spectrometry method for the detection of amitriptyline and nortriptyline by directly injecting human serum and internal standard onto a Cyclone-P online solid-phase extraction (SPE) column (0.5×50 mm). After that analytes were transferred to a Hypersil Gold C-18 analytical column where elution takes place with agradient of water and acetonitrile with 0.1% formic acid. Analytes were ionized and detected over a3.5 min analysis time by electrospray-ionization mass spectrometry with selected reaction monitoring(SRM).

A fast and sensitiveLC–MS/MS method was described by Castroet al. [40] for the simultaneous determination of amitriptyline and nortriptyline in oral fluid and plasma. Chromatographic separation was performed on a Sunfire C18 IS column (20mm×2.1mm, 3.5µm), using agradient of acetonitrile and ammonium formate (pH 3; 2mM) as mobile phase, which allowed the elution of all the compounds in less than 5 min.

LC/MS with sonic spray ionization (SSI) method was described by Shinozukaet al. [41] for the determination of amitriptyline and nortriptyline in the presence of other antidepressants in human plasma. These drug shows good separation and sensitivity by LC–MS using an Inertsil C-8 column with methanol:10mM ammonium acetate (pH 5.0):acetonitrile (70:20:10)as mobile phase at 0.10 mL/min at 35 8C. Solid-phase extraction of these drugs added to the human plasma was performed with an Oasis1 HLB cartridge column. Recovery and limit of detection of these drugs were found to be 69 and 102% and between 0.03 and 0.63 mg/mL, respectively. The proposed procedure was easier and more convenient screening method for antidepressants.

Micellar liquid chromatographic procedure was developed by Boseet al. [42] to determine amitriptyline and nortriptyline in serum samples for Therapeutic Drug Monitoring. Chromatographic determination was carried out using a 0.15 M SDS-6% (v/v) pentanol buffered at pH 7, in a C18 column, followed by electrochemical detection at 650 mV at a flow-rate of 1.5 mL/min having analysis time of 14 min. Limit of detection was found to be 0.25 and 0.31( ng/mL) in serum for amitriptyline and nortriptyline, respectively.

**Table 1. Analytical Techniques for the determination of Amitrityline and Nortriptyline in Pharmaceutical and biological fluids**

| Techniques/ Reagents | Linear Range ($\mu g\ mL^{-1}$) | RSD (%) | Limit of detection ($\mu g\ mL^{-1}$) | References |
|---|---|---|---|---|
| **Spectrophotometric** | | | | |
| 7,7,8,8- tetracyanoquinodimethane | 10 - 300 | 0.21 -0.92 | _ | [13] |
| 3-Methylbenzothiazolin-2-one hydrazone | 1 – 25 and 0.6-30 | 0.04 and 0.035 | _ | [20] |
| Ammonium molybdate and ammonium thiocyanate | 2 - 30 | 0.095–0.627 | _ | [13] |
| Ammonium molybdate | 1 - 140 | 0.35 | 1.0 | [48] |
| Methyl orange | 1 - 25 | 0.13 -0.87 | _ | [15] |
| Bromo cresol green | 1 - 25 | 0.10 -0.59 | _ | [15] |
| 3-Methyl-2-benzothiazolinone hydrazone | 24-216 | 0.64-0.87 | 12 | [17] |
| quinhydrone or *p*-chloranil | 17.8-100 and 25.1-177.8 | 0.15 and 0.55 | - | [18] |
| 7,7,8,8- tetracyanoquinodimethane | 1–10 | - | - | [14] |
| 4-chloro-7-nitrobenzofurazan | 0.4-60 | ≤1.5 | 0.12 | [21] |
| **Spectrofluorimetric** | | | | |
| 9,10-dimethoxyanthracene-2-sulphonate | 0.25–3.00 | 0.40 | 0.30 | [22] |
| 4-chloro-7-nitrobenzofurazan | 0.6-60 | ≤1.5 | 0.18 | [21] |
| capillary gas-liquid chromatography | 0.025-0.250 | 6.2-14.2 and 6.8-15.2 | $1.5\text{-}3.0 \times 10^{-3}$ | [45] |
| capillary zone electrophoresis | 0.16-0.63 and 0.15-0.60 | ≤10 | 0.06 and 0.07 | [23] |
| capillary electrophoresis | 0.01-0.5 | ≤5 | 0.002 | [24] |
| non-aqueous capillary electrophoresis | 0.03-0.50 and 0.03-0.50 | 14.1 | 0.02 | [25] |
| Dispersive liquid–liquid microextraction | 0.005–16 | 5.6–6.4 | 0.005 and 0.01 | [44] |
| liquid chromatography–UV detection | 5.0–1350.0 | 0.07-1.29 | 0.72 | [46] |
| HPLC–MS/ESI | 0.01–3.2 and 0.01-1.0 | 2.8-6.9 and 2.4-5.5 | $3 \times 10^{-3}$ | [32] |
| HPLC | 0.005–5.0 | ≤8 | 0.002 | [31] |
| HPLC-MS | $0.8\text{-}32 \times 10^{-3}$ | - | $0.24 \times 10^{-3}$ | [34] |
| LC-MS | 0.1-1.0 | 2.1 and 5.79 | 0.06 and 0.08 | [41] |
| GC/MS | 0.005-0.1 | 4.1 and 4.2 | 0.7 and 0.30 | [43] |
| Liquid-liquid micro extraction combined with GC | 0.005 - 16 | 5.6 | 0.005 | [47] |

Gas chromatography (GC) and especially GC/MS is a dynamic method for separation and detection of volatile organic compounds. The advent of high- molecular weight products such as polypeptides, or thermally unstable antibiotics limits the scope of this technique. Its principal limitation rests in the relative non-volatility of the drug substances. Therefore, derivatization is mandatory, but the techniques for producing volatile derivatives of drugs are legion. Due to insufficient volatility and thermal stability of the majority of drug materials, gas chromatography can also be used for their assay in a limited number of cases only. Below is an important application of this technique in the field of biological fluids.

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Sensitive gas chromatography–mass spectrometry (GC/MS) method for the determination of amitriptyline and its metabolite nortriptyline in whole blood was described by Papoutsis etal. [43] in whole blood using protriptyline as an internal standard. The combination of solid-phase extraction with derivatization using heptafluorobutyric anhydride efficiently reduced matrix effect and improved sensitivity of the method and later gas chromatography-coupled with mass spectrometry was described by Maresona etal. [49].

## CONCLUSION

In this review, several analytical methods were discussed for the quantitative analysis of amitriptyline and nortriptyline in various pharmaceutical formulations and biological fluids. From the literature survey it was found that mostly Spectrophometric and Spectrofluorimetric methods were employed for the determination in pharmaceutical formulations whereas capillary electrophoresis and chromatographic methods mainly used for the analysis of biological fluids. Capillary electrophoresis is a method which requires low analysis time, small amount of sample and solvent consumption and simple instrumentation. However, due to shorter path length of the flow cell this technique has low sensitivity. Analysis using HPLC is so fast and it is possible to analyse almost all pharmaceutical compounds due to the presence of wide variety of detection system. However instrument is so much costly so it is not possible to afford by each laboratory. Due to easily availability of spectrophotometers in almost all the laboratories, simple procedures involved, economic, speed, accuracy and precision, this technique is still popular and attractive. The data for detection limit, linear range and % RSD of various techniques were presented in Table 1.

## REFERENCES

[1] J.B. Nevado, M.J.V. Lierena, G.G. Cabanillas, V.R. Robledo, S. Buitrago, *J. Sep. Sci.***2006,**29, 103.

[2] T. Shinozuka, M. Terada, E. Tanaka, *Forensic Sci. Int.***2006,** 162, 108.

[3] E. Lacassie, J.M. Gaulier, P. Marquet, J.F. Robatel, G. Lachatre, *J. Chromatogr. B***2000,** 742, 229.

[4] I. Papoutsis, A.Khraiwesh, P. Nikolaou, C.Pistos, C.Spiliopoulou, S.Athanaselis, Journal of Pharmaceutical and Biomedical Analysis**2012,**70, 557.

[5] S.M.R. Wille, K.E. Maudens, C.H. van Peteghem, W.E.E. Lambert, *J. Chromatogr. A***2005,** 1098, 19.

[6] J. Lundmark, M. Reis, F. Bengtsson, *Ther. Drug Monit.***2000,** 22, 446.

[7] M. Espinosa Bosch, A.J. Ruiz Sanchez, F. Sanchez Rojas, C. Bosch Ojeda, *Journal of Pharmaceutical and Biomedical Analysis***2008,** 48, 1290.

[8] M.J. 'O' Neil, *The Merck index,* 14th edn., Merck & Co. Inc., Whitehouse Station, (**2006**).

[9] D. Bose, A. Durgbanshi, A. Matinavarro, M. Capella-Piero, J. Esteve-Romero and M. Gil-Agusti, *J. Pharmacol. Toxicol. Methods* **2005,** 52, 323.

[10] G.E. Schumacher, *Therapeutic Drug Monitoring*, Appleton and large,Connecticut(**1995**).

[11] C.F. Lacy, L.L. Armstrong, N.B. Ingrim and L.L. Lance, *Drug information handbook,* 6th edn., Lexi-Comp Inc., Hudson, Ohio,(**1998-1999**).

[12] *"Martindale The complete drug reference",* 34th edn., Pharmaceutical Press London UK (**2004**).

[13] G.G. Mohamed, F.A.F. Nour El-Dien, N.A. Mohamed, *SpectrochimicaActa Part A***2007,** 68, 1244.

[14] A. Oztunc, N. Dokumacı, E. Tahtasakal, *Il Farmaco***1999,** 54, 835.

[15] F.A.F. Nour El-Dien, G.G. Mohamed, N.A. Mohamed, *SpectrochimicaActa Part A***2006,** 65, 20.

[16] G.G. Mohamed, F.A.F. Nour El-Dien, S.M. Khalil, N.A. Mohamed, *SpectrochimicaActa Part A***2006,** 65, 1221.

[17] N.A. El Ragehy, S.S. Abbas, S.Z. El-Khateeb, *Journal of Pharmaceutical and Biomedical Analysis***2001,** 25, 143.

[18] F.M.A. Attia, IlFarmaco**2000,** 55, 659.

[19] M.A. Moreno , M.P. Ballesteros, P. Frutos, J.L. Lastres, D. Castro, *Journal of Pharmaceutical and Biomedical Analysis*, **2000,** 22, 287.

[20] H.D. Revanasiddappa, B. Manju, *European Journal of Pharmaceutical Sciences***1999,** 9, 221.

[21] L.D.L. Pena, A. Gomez-hens, D. Pirez-Bendito, *Journal of Pharmaceutical & Biomedical Analysis,* **1995**, 13,199.

[22] Maria-Isabel Acedo-Valenzuela, T. Galeano-Dıaz, N. Mora-Dıez, A. Silva-Rodrıguez, *Talanta***2005,** 66, 952.

[23] Shou-Mei Wu, Hsin-Lung Wu, Wei-Kung Ko, Su-Hwei Chen, *AnalyticaChimicaActa***2000,** 413, 125.

[24] Cheng-Chung Chen, Shou-Mei Wu, Yu-HuaHuanga, Wei-Kung Ko, Hwang-Shang Kou , Hsin-Lung Wu, *AnalyticaChimicaActa***2004,** 517, 103.

[25] M.D. Cantú, S. Hillebrand, M.E.C. Queiroz, F.M. Lanças , E. Carrilho, *Journal of Chromatography B,***2004,** 799, 127.

_____

[26] C.D. Aquila, *Journal of Pharmaceutical and Biomedical Analysis* **2002,** 30, 341.

[27] Hwang-Shang Kou, Cheng-Chung Chen, Yu-Hua Huang, Wei-Kung Ko, Hsin-Lung Wu, Shou-Mei Wu**,** *AnalyticaChimicaActa* **2004,** 525, 23.

[28] R. Martino, M. Malet-Martino, V. Gilard, and S. Balayssac, *Analytical and Bioanalytical Chemistry*, **2010,** 398, 77.

[29] I.I. Salim, M. Al-khatib, N. Najib, J. Pharm. Biomed. Anal., **2011**, 56, 983.

[30] L. Chytil, B. Strauch, J. Cvacka, V. Maresova, J.W. Jr, R. Holaj, O.Slanar, J. Chromatogr. B, **2010,** 878, 3167**.**

[31] P. Almudever, J.E. Peris, T. Garrigues, O. Diez, A. Melero, M. Alos, *Journal of Chromatography B*, **2010,** 878, 841.

[32] Y. Shen, Rong-Hua Zhu, Huan-De Li , Yan-Wen Liu, P. Xu, *Journal of Pharmaceutical and Biomedical Analysis* **2010,** 53, 735.

[33] P. Ghahramani, M.S. Lennard, *Journal of Chromatography B,* **1996,** 685, 307.

[34] G. Tybring, J. Nordin, J. Widen, *Journal of Chromatography B*, **1998,** 716, 382.

[35] A. Oztunc, A. Onal, S. Erturk, *Journal of Chromatography B*, **2002,** 774, 149.

[36] H. Kirchherr, W.N. Kuhn-Velten, *Journal of Chromatography B*, **2006,** 843, 100.

[37] M.T.H. Mosavian, Z. Eshaghi, N. Razavi, S. Banihashemi, *Journal of Pharmaceutical Analysis* **2012,** 2, 361.

[38] O.V. Olesen, P. Plougmann, K. Linnet, *Journal of Chromatography B*, **2000,** 746, 233.

[39] A.R. Breaud, R. Harlan, J.M. Di Bussolo, G.A. McMillin, W. Clarke, *ClinicaChimicaActa* **2010,** 411, 825.

[40] A. de Castro, M. Concheiro, O. Quintela, A. Cruz, M. Lopez-Rivadulla**,** *Journal of Pharmaceutical and Biomedical Analysis* **2008,** 48, 183.

[41] T. Shinozuka, M. Terada, E. Tanaka, *Forensic Science International* **2006,** 162, 108.

[42] D. Bose, A. Durgbanshi, A. Martinavarro-Domınguez, Maria-Elisa Capella-Peiro, S. Carda-Broch, J. Esteve-Romero, M. Gil-Agustı, *Journal of Pharmacological and Toxicological Methods* **2005,** 52, 323.

[43] I. Papoutsis, A. Khraiwesh, P. Nikolaou, C. Pistos, C. Spiliopoulou, S. Athanaselis, *Journal of Pharmaceutical and Biomedical Analysis* **2012,** 70, 557.

[44] A.S.Yazdi, N.Razavi, S. R.Yazdinejad, *Talanta*, **2008,** 75, 1293.

[45] S. Ulrich, T. Isensee , U. Pester, *Journal of Chromalography B,* **1996,** 685, 81.

[46] S.Ashour, N. Kattan, *J. Pharmaceutical Analysis*, 2012,2,437.

[47] A. S. Yazdi, N. Razavi, S.R. Yazdinejad, *Talanta,* **2008**,75 () 1293.

[48] T. Aman, A.A. Kazi, M.I. Hussain, S. Firdous, I.U. Khan, *Anal. Lett.* **2000**, 33, 2477.

[49] V. Maresona, J. Chadt, I. Novakova, *Neuro End. Lett.* **2008***,* 29, 749.