ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4

Journal of Coastal Life Medicine

Quantitative Determination of Amitriptyline by GC-FID Using Derivatization Method

Received: 16 February 2023, **Revised:** 19 March 2023, **Accepted:** 17 April 2023

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Keywords
Gas chromatography, Amitriptyline Hydrochloride, Ethyl acetate, Flame ionization detector (FID)

Abstract
A novel and new gas chromatographic technique has been developed for the quantitative determination of Amitriptyline in bulk drug as well as pharmaceutical dosage form. Ethyl acetate was used as a precolumn derivatizing agent. The separation by GC was carried out by using 10% OV-17 column (bonded and crosslinked 50% diphenyl 50% dimethylpolysiloxane) in combination with flame ionization detector (FID). Nitrogen was used as carrier gas. For the elution, the initial temperature of oven was maintained at 1000 C for 1 min. and then the temperature was increased at the rate of 100 C per min. The temperature of detector and sample injector were kept isothermal at 2000 C. Various parameters were used for the validation of developed method. The calibration curve was found to be linear in the concentration range of 1-10 μg/ml. and correlation coefficient (r2) value was found to be 0.9984. The % assay was found to be 99.32% with standard deviation of 0.0402 and % RSD value of 0.4050. The developed method is highly precise, specific, accurate and reproducible and could be applied for derivatization and estimation of amitriptyline in its pure and pharmaceutical dosage form.

1. Introduction

Amitriptyline is a tricyclic antidepressant which is used for the treatment of depressive illness, which can be either endogenous or psychotic, and to relieve depression associated anxiety. It is widely used to treat neuropathic pain and depression. [1] Physically, amitriptyline is a white coloured, crystalline compound which is freely soluble in water. [2]

The mechanism of action of this drug is not fully known. It is said that amitriptyline acts by inhibiting the membrane pump mechanism which is responsible for the re-uptake of transmitter amines, such as norepinephrine and serotonin, which then thereby

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increase their concentration at the synaptic clefts of the brain. These amines are important in mood regulation. [3,4]

According to a literature, a method of GC-MS was developed for estimation of amitriptyline with other drugs in pure and biological fluids. The chromatographic detection was performed by MS detector and HP-5MS column. Helium was used as carrier gas. The retention time for amitriptyline was observed to be 5.75 minutes. [5]

Another method was developed for analysis of amitriptyline in plasma and urine by using electro membrane sample preparation method where they have used HP-5 column, FID detector and helium as carrier gas. [6]

According to a method, amitriptyline was analyzed in the blood by using GC-MS method and helium as carrier gas. [7]

2. Methodology

Chemicals, solvents and reagents:

All the solvents used for GC were of HPLC grade which were initially sonicated for 20 minutes and then filtered to remove any particulate present.

Sample preparation and derivatization:

Amitriptyline hydrochloride pure drug was acquired. The pure drug was made volatile and converted to its ester form using ethyl acetate as a derivatizing agent. Initially, amitriptyline hydrochloride (10 mg) was dissolved in appropriate diluent (10 ml) which is considered as aqueous phase. Then ethyl acetate (5ml) was dissolved in methylene chloride (5 ml) separately, which is considered as organic phase. Equal volume of aqueous (5 ml) and organic (5 ml) phases were taken and shaken together in centrifuge tube at 200C for 20 min. the lower organic phase was separated and added to another flask containing toluene (0.5 ml). The organic phase was then reduced to 0.5 ml by evaporation. Few millilitres of this organic phase were taken for analysis by GC. Dilutions were prepared by using methanol as diluent for further validation parameters. [8,9]

Chromatographic conditions:

GC studies were carried out on IA-2100 model 2020 coupled with flame ionization detector (FID). A computer having N2000 online chromatostation software has been used to monitor the gas chromatograph. 10% OV-17 column (bonded and crosslinked 50% diphenyl 50% dimethylpolysiloxane) with 30 meters of length and 0.25 mm of internal diameter was used for the whole study.

The air pressure was maintained at 0.2 kg/m2. Pressure of hydrogen and carrier gas i.e., nitrogen was maintained at 1 kg/m2. All the gases which were used in this study were of pharmacopeial purity. The injector port and detector temperature were maintained at 2000 C. Various trials of different temperature programmes were performed for GC oven. Best programme was selected for a good resolution at the end of the study. Manual splitless injection of 2 µL was used for insertion of sample into instrument. The initial temperature of oven was maintained at 1000 C for 1 min. and then the temperature was increased at the rate of 100 C per min. [10]

Analytical method development:

Analytical method development is the consistant process that arises in parallel with development of pharmaceutical product. Suitable method development is essential when talking about cost, time, productivity, and effectiveness of drug product. Analytical method development is essential for drug degradation studies, analyzing and evaluating properties of API, and to study impurities in the drug. [11]

Method validation:

Method validation is the process intended to check whether the method of analysis shows appropriate acceptable measurements as per the regulations. The method was validated for various parameters such as, linearity, precision, accuracy, and recovery according to ICH guidelines. [12,13]

Assay:

Assay is the quantitative estimation of content of sample drug in comparison with standard. 20 tablets of amitriptyline marketed formulation were triturated in mortar. Amount equivalent to 10 mg amitriptyline was taken from triturated tablet powder. It was then transferred to 100 ml volumetric flask. 20 ml of diluent was added and then woke up the volume upto 100 ml

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by suitable solvent. From this stock solution, further dilutions were made. [14]

Linearity:

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration of analyte in the sample. [15] The linearity was determined by constructing calibration curve. The derivatized sample of different concentration in the range of 1-10 µg/ml were used for the analysis. Three replicates were used for each measurement. Linear regression equation (y = mx + c) and the correlation coefficient (r2) of the calibration curve was obtained by plotting the peak area versus actual concentrations. Using regression equation, the values of concentrations were statistically evaluated.

Accuracy:

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or reference value and the value obtained by the method. Three different levels 80%, 100% and 120% of the label claim were used in triplicate for the determination of accuracy. [16]

Precision or repeatability:

Precision of an analytical procedure is the degree of agreement between individual test results which are obtained by the method. [17]

Intra- Day Precision:

Six replicates of sample solutions were prepared representing a single batch and injected as per analytical method.

Inter-Day Precision:

Six replicates of sample solutions were prepared representing same batch (same batch taken for intraday precision) on a different day i.e., for 1st & 2nd day as per the analytical method. [18]

LOD and LOQ:

The term LOD is the lowest concentration which can be detected. It is calculated by using the equation, LOD = 3.3 × σ/S.

The term LOQ is the lowest concentration which can be quantified. It is calculated by using the equation, LOQ = 10 × σ/S.

where σ represents the residual standard deviation of the regression line and S represent the slope of the corresponding regression line. [19]

3. Result:

Method development and optimization:

Amitriptyline is tertiary amine. For GC, this tertiary amine was derivatized by ester and converted into volatile. Organic solvent i.e., methylene chloride containing ethyl acetate was used for the extraction of amine. This derivatization method led to formation of new compound having high volatility which is required for GC.

The chromatographic conditions were optimized by performing several trials to achieve a good sensitivity, resolution, and symmetric peak of analyte. Several temperature programs as well as different flow rates of carrier gas (nitrogen) were tried to achieve maximum selectivity and sensitivity of the method. Sharp peak of amitriptyline was obtained by using 10% OV-17 column and flame ionization detector. The applied temperature and pressure program and detailed analytical parameters are mentioned previously in the 'chromatographic conditions' section. The retention time (RT) was found 0.92 min.

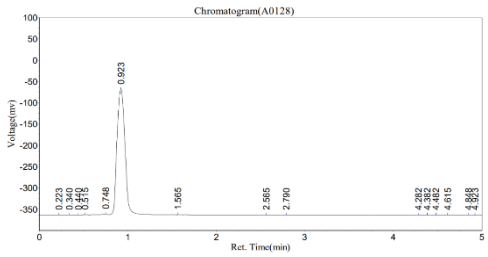

Fig.1 GC-FID chromatogram of standard amitriptyline at 10 µg/ml after derivatization

Method validation:

Linearity:

The regression equation was obtained by plotting the calibration curve of peak area versus concentration of analyte. The linear regression equation for calibration curve was found to be y=185655x+9886.9 and the

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ISSN: 2309-6152 (Online)
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correlation coefficient (r2) was found as 0.9984.

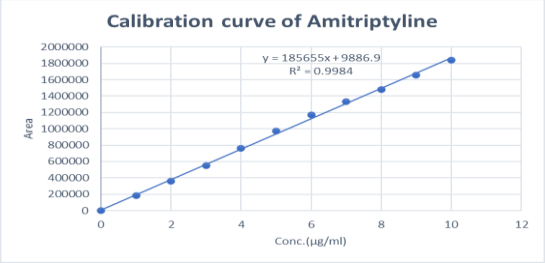

Fig.2 Calibration curve of standard amitriptyline in range of 1-10 µg/ml

Thus, the results of the calibration curve showed good linearity having an acceptable correlation coefficient within the concentration range of 1–10 µg/ml. All the calculated values are mentioned in the table 1.

Table 1. Summary of calibration standards

Sr. No.	Conc. (µg/ml)	Area
1	1	185234.4
2	2	362577.9
3	3	551789.2
4	4	763486.3
5	5	972718.6
6	6	1170347.8
7	7	1330978.2
8	8	1483978.3
9	9	1659756.2
10	10	1838898.0

Specificity:

Amitriptyline in the concentration of 10 µg/ml was prepared from standard stock solution. The peak was observed at the retention time of 0.92 min. There were no interfering peaks at or near the area of the peak of interest in the chromatogram, which shows the high specificity of the method.

Precision (Repeatability):

Precision was determined as intra-day (within a single day) and inter-day (between three days). Six replicates of same concentration i.e., 10 µg/ml were analyzed in one day for intra-day precision and the samples were analyzed for three consecutive days for inter-day precision. All the calculated values are mentioned in the table 2.

Table 2. Summary of Intra-day and Inter-day precision

Level	Conc. added (µg/ml)	Conc. found by graph (µg/ml)	SD	% RSD	n
Intra-day	10	9.94	0.0783	0.7877	6
Inter-day D-1 D-2 D-3	 10 10 10	 9.97 10 9.99	 0.0682 0.1021 0.0783	 0.6838 1.021 0.7835	 6 6 6

Accuracy:

The accuracy of the developed method was estimated as % recovery. The accuracy was determined at three different levels 80%, 100% and 120% of the label claim. Six replicates of same concentration i.e., 10 µg/ml were analyzed. All the calculated values are mentioned in the table 3.

Table 3. Summary of accuracy

Accuracy	Amount added (mg)	Amount recovered (mg)	% Recovery	n
80%	8	7.95	99.37	6
100%	10	9.95	99.56	6
120%	12	11.94	99.52	6

Assay:

The assay was determined as the % drug content. 6 dilutions of concentration 10 µg/ml were made from

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this stock solution. The % drug content of was found to be 99.32 %. All the calculated values are mentioned in table 4.

Table 4. Summary of assay

Label claim (mg)	Drug content found (mg)	% Drug content	SD	% RSD	n
10	9.93	99.32	0.0402	0.405	6

4. Discussion

All the results found within the limit. The correlation coefficient (r2) was found to be not less than 0.99. %RSD for precision was found to be not more than 2. The mean recovery at all three levels was found to be not less than 98% and not more than 102%. The assay was found to be not less than 98% and not more than 102%.

5. Conclusion:

A simple, rapid, linear, reliable, specific, and reproducible GC-FID method for estimation of amitriptyline in pure drug as well as pharmaceutical dosage form i.e., tablet was developed and validated. A new rapid and simple method was developed for derivatization of amitriptyline. The method reported in the present work has been effectively and efficiently used to analyze amitriptyline without any interference from the other excipients. Therefore, this GC-FID method can be used for the routine QC analysis of amitriptyline in its API as well as pharmaceutical preparations.

References:

[1] W. A. Garland, R. R. Muccino, et.al. A method for the determination of amitriptyline and its metabolites nortriptyline, 10-hydroxyamitriptyline, and 10-hydroxynortriptyline in human plasma using stable isotope dilution and gas chromatography-chemical ionization mass spectrometry (GC-CIMS). Clin. Pharmacol. Ther., 1979, 844-856.

[2] Anbessa Bekela. Analytical Methods for Amitriptyline: A Review. International Journal of Scientific & Engineering Research. Volume 11, Issue 4. 2020, 742-768.

[3] Amit Thour, Raman Marwaha. Amitriptyline. Europe PMC. StatPearls Publishing. 2022.

[4] Kjell Fuxe, Sven-ove Ogren, et.al. On the mechanism of action of the antidepressant drugs amitriptyline and nortriptyline. Evidence for 5-Hydroxytryptamine receptor blocking activity. Neuroscience Letters. Volume 6 1977. 339-343.

[5] Ibrahim A Naguib, Nesma A Ali, et.al. US FDA-validated green GC–MS method for analysis of gabapentin, tramadol and/or amitriptyline mixtures in biological fluids. Bioanalysis. Newlands press, 2020, 0217.

[6] Rezazadeh M., Yamini Y., et.al. Electromembrane surrounded solid phase microextraction: A novel approach for efficient extraction from complicated matrices. J. Chromatogr. A 2013, 1280, 16–22.

[7] Paterson S, Cordero R, Burlinson S. Screening and semi-quantitative analysis of post mortem blood for basic drugs using gas chromatography/ion trap mass spectrometry. J. Chromatogr. B 2004, 813, 323–330.

[8] Karl-Erik Karlson. Two phase derivatization of amitriptyline and structurally related tertiary amines for gas chromatography with electron-capture detector. Journal of Chromatography, Elsevier Scientific Publishing Company. 219 1981. 373-378.

[9] Danny Rich, Jen Green, et.al. Development of a new “Fast GC-Ms” system for multi-drug analysis. Journal of Association of Public Analysts, 2016, 001-011.

[10] Natalia Manousi, Victoria F. Samanidou. Applications of Gas Chromatography for the Analysis of Tricyclic Antidepressants in Biological Matrices. Separations. 2019. 6. 24.

[11] Neera Chikanbanjar, Nidhi Semwal, et.al. A review article on analytical method validation. Journal of Pharma Innovation. Volume 1, Issue 1. 2020. 48-58.

[12] Pedro Araujo. Key aspects of analytical method validation and linearity evaluation. Journal of Chromatography B. 877. 2009. 2224-2234.

[13] Dennis R. Jenke. Chromatographic method validation: a review of current practices and procedures. 2. Guidelines for primary validation parameters. Journal of Liquid Chromatography & Related Technologies. 19(5). 1996. 737-757.

[14] Neeli Sujatha, K. Haritha Pavani. Analytical method development and validation of

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

amitriptyline hydrochloride and chlordiazepoxide in tablet by rp-hplc. Indian Journal of Research in Pharmacy and Biotechnology. 2013. 1(5). 655-659.

[15] Lawanya Chowdary G., Ravisankar P., et.al. Analytical method validation parameters: An updated review. International Journal of Pharmaceutical Sciences Review and Research. 61 (2). 2020. 1-7.

[16] G. Geetha, Karanam Naga Ganika Raju, et.al. Analytical method validation: An updated review. International Journal of Advances in Pharmacy, Biology and Chemistry. Volume 1 (1). 2012. 64-71.

[17] Panchumarthy Ravisankar, Ch. Naga Navya, et.al. A review on step-by-step analytical method validation. IOSR Journal of Pharmacy. Volume 5, Issue 10. 2015. 07-19.

[18] Linnea D. Duke. Method validation for amitriptyline and norytiptyline in artificial foodstuffs. Canadian police research centre. 2004. 27-39.

[19] Shipra Thapar, Anshul Chawla, et.al. A concise review on method development and validation parametrs. GSC Biological and Pharmaceutical Sciences, 19 (01), 2022, 066-077.