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Research Article

QUANTITATIVE DETERMINATION OF LEVETIRACETAM BY GAS CHROMATOGRAPHY USING ETHYL CHLOROFORMATE AS A DERIVATIZING REAGENT IN PURE AND PHARMACEUTICAL PREPARATION

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ABSTRACT

A novel gas liquid chromatographic (GC) method has been developed for the quantitative estimation of Levetiracetam (LEV) in bulk drug and pharmaceutical dosage forms. Ethyl chloroformate (ECF) was used as a precolumn derivatizing reagent. GC separation was carried out on an Rtx-5 capillary column (cross bond 5% diphenyl/ 95% dimethyl polysiloxane) with a length of 30 meters and an internal diameter of 0.25 mm with flame ionization detector. The elution was carried out at an initial temperature of 80˚ C for 4 minutes and temperature increased at the rate of 100˚C/min up to 180˚C for 5 min. Column pressure was programmed as 29.8 Kpa for 3.5 minutes and pressure increased at rate of 20Kpa/min up to 120Kpa for 4.50 minutes. The linear calibration ranges for LEV was observed between 2- 10 ng/ml. The method was subsequently applied to the determination of LEV in pharmaceutical preparations. The relative standard deviation (RSD) was found to be 0.122**%.** The recovery studies were done and the percentage recovery of LEV was found to be 101.76%.

Keywords: Ethyl chloroformate (ECF), flame ionization detector (FID), gas chromatography, levetiracetam (LEV).

INTRODUCTION

Levetiracetam[1] (LEV) is a novel antiepileptic agent; with a chemical name (S)-(2)-(2-oxopyrrolidin-yl) butamide. The chemical strucuture of levetiracetam was shown in Fig.1. It is used as an adjunctive therapy in the treatment of partial seizures[2]. Levetiracetam can prevent myoclonic jerks and generalized epileptiform

activity in patients with photosensitive epilepsy. The precise mechanism by which levetiracetam exerts its antiepileptic effect is unknown. However the drug binds to a synaptic vesicle protein[3], which is believed to impede nerve conduction across synapses [4].

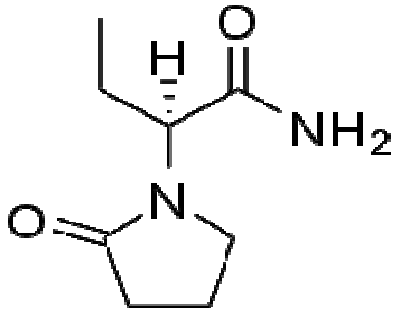


Fig-1: Structure of Levetiracetam

The therapeutic importance of LEV was behind the development of numerous methods for its determination. Literature survey reveals that various HPLC[5-10] and LC-MS [11-13] methods have been reported for the determination of levetiracetam in pure and pharmaceutical dosage forms. These methods require long and tedious pre-treatment of the samples and laborious clean up procedures prior to analysis. An official monograph of LEV does not exist in any pharmacopoeia and determination of LEV in bulk and pharmaceutical formulations has not been yet described. A through literature search has revealed that no gas chromatographic methods available for determination of pregabalin in bulk drugs and pharmaceutical formulations. So there is a lot of scope for development of suitable gas chromatography method for the determination of LEV in bulk and pharmaceutical formulations. The present work examined the capillary GC determination of LEV after derivatization with ethyl chloroformate (ECF)[14] with flame ionization detector (FID) determination of the drug. The method reported here is sensitive, reproducible and rapid, which is suitable for the accurate determination of LEV in pure and pharmaceutical dosage form.

EXPERIMENTAL

Reference Standards, Reagents, Preparations

Analysis was performed on methanol solutions of LEV. All the standards were supplied by Sigma and met Pharamcopoeial requirements. Methanol of analytical quality was procured from MERCK (Worli, Mumbai).

Levetiracetam solution at a concentration of 1 mg/ml was prepared in HPLC grade methanol and diluted to obtain serial dilutions from 2 to 10 ng/ml. The solutions were kept at below 5˚C and were protected from light.

The studies were conducted on the pure drug LEV and formulation TORLEVA 250 (250mg, torrent). An amount of powder equivalent to the average weight of a tablet was mixed with methanol and shaken for 20 min at a frequency of approximately 3 cycle's/s in sonicator. The solution was then filtered through 0.45 μm membrane filter. The drug solution and ECF (98%) were added in 1:1 ratio. The solution was heated at 70^0 C for 5 minutes. Solution was evaporated after addition of pyridine and chloroform. Upper layer was collected, redissolved in methanol.

Chromatographic conditions

GC studies were carried out on SHIMADZU model 2014 (Shimadzu Technologies, Japan) coupled with a split/split less injector, operated in a split-mode and FID. The computer with GC solutions software has been used to control the gas chromatograph. Rtx-5 capillary column (cross bond 5% diphenyl/95% dimethyl polysiloxane) with a length of 30 meters and an internal diameter of 0.25 mm was used throughout the study.

The GC-FID parameters used in the method development were based on the boiling point of the drug. The injection port and detector temperature were set to 170˚C and 250˚C, respectively. Different temperature programs were investigated for GC oven. Best program temperature (180˚C) resolution was selected for a good resolution at end of the investigation.

Manual splitless injection of approximately 2-μl sample was performed at an inlet temperature of 170˚C. The detector temperature was set to 250˚C. After injection, the oven temperature was increased quickly from 80˚C to 180˚C, and then programmed within 5 min to 180˚C at a rate of 100˚C per min for 5min. The initial pressure was maintained at 29.8 Kpa for 3.5 minutes and pressure was increased at a rate of 20Kpa/min up to 120Kpa and held constant for 4.50 min.

Nitrogen at a flow rate of 0.8 ml/min was used as a carrier gas. Synthetic air (flow rate of 100 ml/min), hydrogen (25 ml/min) were fed to the FID. All the gases used in these studies were of Pharmacopoeial purity.

LEV analysis was performed after derivatization, LEV is a polar molecule and therefore, a polar solvent methanol was used as the diluent. The capillary column coated with 5% diphenyl/ 95% dimethyl polysiloxane is a good choice for separation of this analyte since they elute as symmetrical peaks at a wide range of concentrations.

Method validation

After the method conditions were established as described above, method was validated for precision, accuracy, and linearity. Precision was measured as the repeatability of a series of results (*n*=6) and was also checked inter-day. Accuracy was determined as percentage recovery (*n*=3) at three concentrations (80,100 and 120% of the amount expected) achieved by spiking placebo with reference standard. Linearity was established by chromatography of a series of solutions (*n*=5) of decreasing concentrations. The limit of detection (LOD) and quantification (LOQ) were determined. The results are shown in table 1. Quantative analysis of the drugs was performed under the conditions established. The selectivity of the method was evaluated by comparing retention time values in chromatograms obtained from the analyzed product with those in the chromatograms obtained from reference standard. There were no additional peaks found in the chromatogram.

Table-1: Validation report for gas chromatograph for determination of Levetiracetam.

Parameter	Value
Linearity(ng)	2-10
LOD (ng)	0.016
LOQ (ng)	0.051
Recovery %	101.76

The linearity of peak area response versus concentration for LEV was studied between concentration ranges of 2-10 ng/ml. The calibration curve constructed was evaluated by its correlation coefficient. The calibration equation from six replicate experiments, demonstrated the linearity of the method.

The precision of the analytical method was determined by repeatability (intra-day) and intermediate precision (inter-day). Three different concentrations which were QC samples (2, 4, 6 ng/ml) were analyzed six time in one day for intra-day precision and once daily for three days for inter-day precision. The RSD value for intra-day precision was 0.122% and for inter-day precision was 0.132%. These values are summarized in Table 2.

Table-2: Precision and accuracy of LEV by GC-FID method.

Parameters	LEV	
	Intraday	Interday
Mean	87313431.6	89552338.17
S. D	107286.6	1118997.7
%RSD	0.12	0.13

To determine the accuracy of the proposed method and to study the interference of formulation additives, the recovery was checked at three different concentration levels (2, 4, 6 ng/ml) and analytical recovery experiments were performed by adding known amount of pure drugs to pre-analyzed samples of commercial dosage form. The percent analytical recovery values were calculated by comparing concentration obtained from the spiked samples with actual added concentrations. These values are also listed in Table 3.

Table-3: Recovery values of LEV by GC-FID method in pharmaceutical preparations.

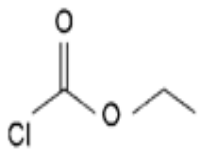
Commercial preparation		LEV tablet	
Method	**Added (ng ml^{-1})**	**Recovery (%)**	**RSD% [a]**
GC-FID	2	96.74	3.57
	4	98.88	3.32
	6	97.46	4.36

SD: Standard deviation of six replicate determinations, RSD: Relative standard derivation
a: average of six replicate determinations

RESULTS AND DISCUSSION

The compound LEV reacted with ECF to form a volatile product (Fig.2.), and eluted from a capillary GC column, each having a single peak. The reaction was carried out in methanol. A better GC response (average peak height/peak area) was observed using an aqueous solution containing pyridine as the reaction medium. The effect of pH on the derivatization was examined between 1-10 at unit interval. It was observed that derivatization occurred at pH value above 6. The reaction mixture was sonicated at room temperature (30˚C) for 5-20 min at an interval of 5 min and the optimum response was observed within 15 min.

(a)



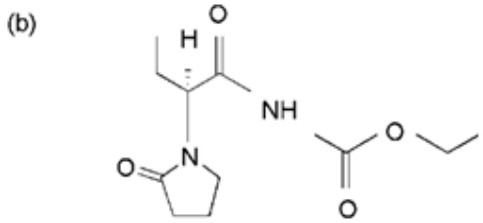


Fig.2. Structure diagram of the derivative ECF (a) ECF (b) LEV

Individual chromatograms were recorded for LEV. Chromatograms obtained for LEV are shown in Fig-3. On the basis of the chromatograms obtained, characteristic retention times were determined for the drug as the basis for qualitative identification (Table 4). The chromatogram obtained for the tablet sample is shown in Fig-4. Blank chromatogram was shown in Fig-5.

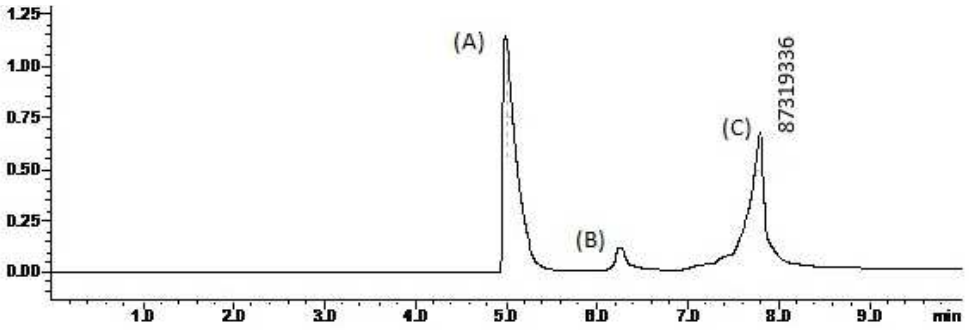


Fig-3: Chromatogram obtained from pure Levetiracetam (LEV) solution, Methanol (A), ethyl chloroformate (B), levetiracetam (C)

Table-4: Relative retention time.

Compound name	Retention time in minutes		Relative retention time
	Standard	Sample	
Levetiracetam	7.816	7.886	0.991

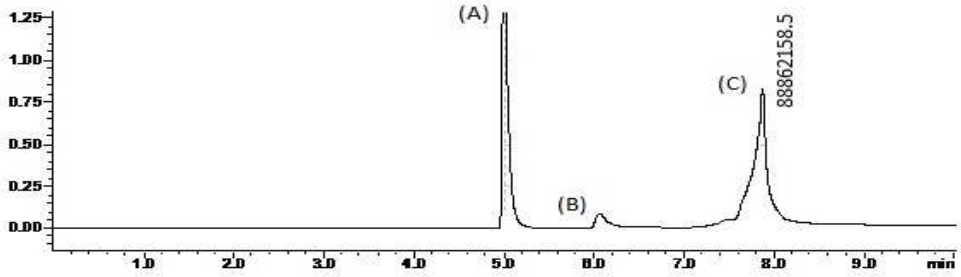


Fig-4: Chromatogram obtained from formulation of Levetiracetam (LEV) tablet solution. Methanol (A), Ethyl chloroformate (B), levetiracetam (C).

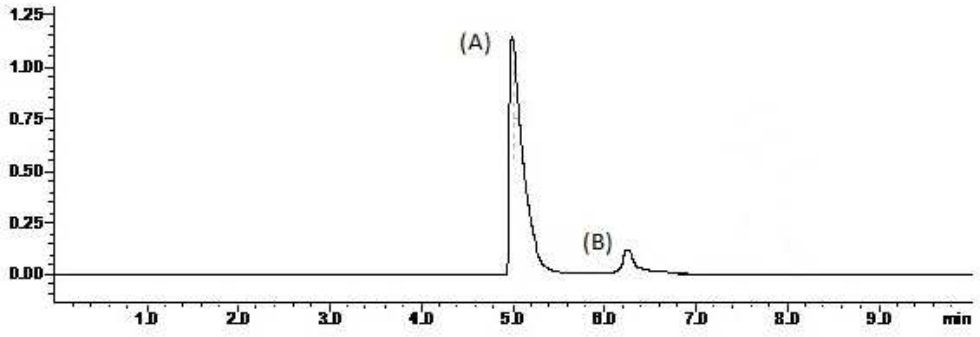


Figure-5: Blank Chromatogram, Methanol (A), ethyl chloroformate (B).

CONCLUSION

In the present report, a simple, rapid, sensitive, reliable, specific, accurate and precise GC-FID method for the determination of LEV in pharmaceutical preparation was developed and validated. The method described in the present report has been effectively and efficiently used to analyze LEV pharmaceutical dosage form without any interference from the pharmaceutical excipients. Therefore, GC-FID method can be used for the routine QC analysis of LEV in pharmaceutical preparations.

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