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

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ABSTRACT
A novel Gas Chromatographic (GC) method has been developed for the quantitative estimation of Lamotrigine (LMT) 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 LMT was observed between 2-10 ng/ml. The method was subsequently applied to the determination of LMT in pharmaceutical preparations. The relative standard deviation (RSD) was found to be 0.17%. The recovery studies were done and the percentage recovery of LMT was found to be 97.86%.

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Please cite this article in press as R S Chandan et.al. Quantitative determination of lamotrigine by gas chromatography using ethyl chloroformate as a derivatizing reagent in pure and pharmaceutical preparation. Indo American Journal of Pharm Research.2013:3(10).

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INTRODUCTION
Lamotrigine (LMT), 3, 5-diamino-6-(2, 3-dichlorophenyl)-1, 2, 4-triazine (Figure 1) is a new generation antiepileptic drug registered for treatment of patients with refractory partial seizures with or without secondary generalization [1, 2]. It acts by inhibiting presynaptic voltage-sensitive sodium channels and excitatory neurotransmitter release.

The mechanism of action of lamotrigine is inhibition of the release of excitatory neurotransmitters (aspartate and glutamate) and also involvement of the blocking of voltage dependent sodium channels [3]. Lamotrigine is effective for treatment of partial and generalized tonic, clonic seizures as a single drug or as an adjuvant with other anti epileptic drugs [4]. The aim of the present study was to develop and validate a simple, gas liquid chromatographic method for the determination of lamotrigine in tablets. The developed method was validated using ICH guidelines for validation.

HPLC [5-8] and capillary electrophoresis [9, 10] and few spectrophotometric methods [11-12] are used for the measurement of LMT concentrations in pharmaceutical products and biological fluids. LMT in human plasma has been assayed using solid phase micro extraction (SPME) and gas chromatography with thermionic specific detection [13] whereas in serum it has been determined by high performance thin layer chromatography [14]. A through literature search has revealed that no gas chromatographic methods available for determination of lamotrigine in bulk drugs and pharmaceutical formulations. So there is a lot of scope for development of suitable gas liquid chromatography method for the determination of LMT in bulk and pharmaceutical formulations. The present work examined the capillary GC determination of LMT after derivatization with ethyl chloroformate (ECF) (Figure 1) [15] with flame ionization detector (FID). The method reported here is sensitive, reproducible and rapid, which is suitable for the accurate determination of LMT drug.

MATERIALS AND METHODS
Reference Standards, Reagents, Preparations
Analysis was performed on methanol solutions of LMT. All the standards were supplied by Sigma and met Pharmacopoeial requirements. Methanol of analytical quality was procured from MERK (Worli, Mumbai).

Lamotrigine 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 low temperatures and were protected from light.

The studies were conducted on the drug lamotrigine and formulation LAMITOR OD with a labelled strength of 50 mg and manufactured by Torrent Pharmaceuticals Limited, Himachal Pradesh, India. An amount of powder equivalent to 10mg was mixed with methanol and shaken for 20 min at a frequency of approximately 3 cycle’s/s in a sonicator. The solution was then filtered through 0.2 μm whatman filter paper. The drug solution and ECF (98%) were added in 1:1 ratio. The solution was heated at 70°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 and 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. Lamotrigine has a boiling point of about 210-219˚C. 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 4 min to 180˚C at a rate of 100˚C per min for 5 min. 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.
LMT analysis was performed after derivatization. LMT 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. These values are summarized in Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity (ng)</td>
<td>2-10</td>
</tr>
<tr>
<td>LOD (ng)</td>
<td>0.0116</td>
</tr>
<tr>
<td>LOQ (ng)</td>
<td>0.0352</td>
</tr>
<tr>
<td>Recovery (%)</td>
<td>97.58</td>
</tr>
</tbody>
</table>

Quantitative 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.

The linearity of peak area response versus concentration for LMT 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, \(y = 2500006.5x + 931109\) \((r = 0.997)\), 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 0.17% and for inter-day precision was 0.25%. These values are summarized in Table 2.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>LMT</th>
<th>LMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>50347395</td>
<td>50267944</td>
</tr>
<tr>
<td>S. D</td>
<td>88032.9160</td>
<td>126277.6177</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.17</td>
<td>0.25</td>
</tr>
</tbody>
</table>

To determine the accuracy of the proposed method and to study the interference of formulation additives, the recovery was checked as 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>
<thead>
<tr>
<th>Commercial preparation</th>
<th>LMT tablet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method</td>
<td>Added (ng ml(^{-1}))</td>
</tr>
<tr>
<td>GC-FID</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>6</td>
</tr>
</tbody>
</table>

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

\(^a\): average of six replicate determinations
RESULTS AND DISCUSSION

The drug LMT reacted with ECF to form a volatile product (Figure 1), 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.

Individual chromatograms were recorded for LMT. Examples of chromatograms obtained for LMT are shown in Figure 2, respectively. 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 Figure 3 and blank chromatogram is shown in Figure 4.

![Figure 1: Structure diagram of the derivative ECF (a) ECF (b) LMT](image)

![Figure 2: Chromatogram obtained from pure Lamotrigine (LMT) solution, Methanol (A), ethyl chloroformate (B), lamotrigine (C)](figure)

![Figure 3: Chromatogram obtained from formulation of Lamotrigine (LMT) tablet solution, Methanol (A), ethyl chloroformate (B), lamotrigine (C)](chart)
Figure 4: Blank Chromatogram, Methanol (A), ethyl chloroformate (B)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention time in minutes</th>
<th>Relative retention time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard</td>
<td>Sample</td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>7.856</td>
<td>7.928</td>
</tr>
</tbody>
</table>

CONCLUSION

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

ACKNOWLEDGEMENTS

The authors express their sincere thanks to Torrent Pharmaceuticals Limited, Himachal Pradesh, India for supplying the gift sample of lamotrigine. Authors also extend their thanks to the Principal, JSS College of Pharmacy, Mysore and JSS University, Mysore for providing the facilities to carry out the research work.

AUTHORS’ STATEMENTS

Competing Interests

The authors declare no conflict of interest.

REFERENCES