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Short communication

A stability-indicating liquid chromatographic method for Lomustine

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ABSTRACT

A simple, inexpensive and rapid liquid chromatography (LC) method has been developed for the quantitative determination of Lomustine, an chemotherapy drug. Degradation studies were performed on the bulk drug by heating to 60 °C, exposure to UV light at an energy of 200 Wh/m^2 and to visible light at an illumination of not less than 1.2 million lux hours, acid (0.1N hydrochloric acid), base (0.1N sodium hydroxide) aqueous hydrolysis and oxidation with 6.0% (*v*/*v*) hydrogen peroxide. Good resolution between the peaks corresponding to impurities produced during synthesis, degradation products and the analyte was achieved on a Symmetry C 8 LC column using a mobile phase consisting of a mixture of aqueous potassium dihydrogen phosphate and acetonitrile. The degradation samples were assayed against the reference standard of Lomustine and the mass balance in each case was close to 99.9%. Validation of the method was carried out as per International Conference on Harmonization (ICH) requirements.

1. Introduction

Lomustine, *N*-(2-chloroethyl)-*N*′-cyclohexyl-*N*-nitrosourea, is an alkylating agent of value against both hematologic malignancies and solid tumors developed by Bristol Myers Squibb. The empirical formula for Lomustine is $C_9H_{16}ClN_3O_2$, and the molecular weight is 233.695 The International Conference on Harmonization (ICH) guidelines [1] require stress testing of drug substances. Moreover, a validated analytical method must be applied in stability studies [2].

So far analytical method for Lomustine was mentioned in European Pharmacopeia [3]. But for the related substances TLC method was followed. The other literature methods [4,5] discussed about quantification of Lomustine and its metabolites but not the related substances. As the compound and the related substances are non-UV active, development of stability-indicating LC method was a challenging task. The current work deals with the accelerated degradation of the drug substance under stress conditions like hydrolysis, oxidation, thermal and UV light. The work also includes the validation of the stability-indicating method developed.

2. Experimental

2.1. Chemicals and reagents

Samples of Lomustine and its three impurities Imp-A, Imp-B and Imp-C (Fig. 1) were received from the Process Research Department of Integrated Product Development Operations of Dr. Reddy's Laboratories, Hyderabad, India. LC grade acetonitrile, potassium dihydrogen orthophosphate and phosphoric acid were purchased from Merck, Schuchardt, Germany. Pure water was prepared by using a Millipore Milli Q plus purification system (Bedford, MA, USA).

2.2. Instrumentation

The LC system was a Waters model 2996 equipped with a PDA (Waters Corporation, Milford, USA). The output signal was monitored and processed using Empower software (Waters Corporation, Milford, USA) on a Pentium computer (Digital Equipment Co.).

2.3. Chromatographic conditions

Chromatographic separation was achieved on a 5-μm Symmetry C18 column (250 mm × 4.6 mm), using a mobile phase with a buffer containing a mixture of 0.01 M aqueous potassium dihydrogen orthophosphate, pH adjusted to 3.0 using dilute phosphoric acid. The mobile phase A is a mixture of buffer and acetonitrile (80:20, *v*/*v*), and mobile phase B is a mixture of buffer and acetoni-

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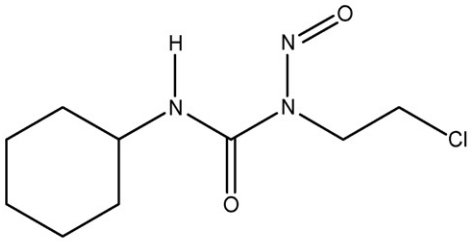
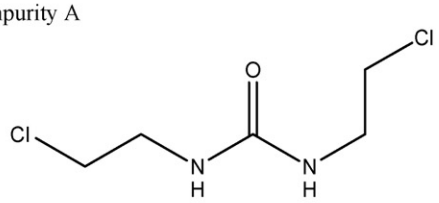
(a) Lomustine



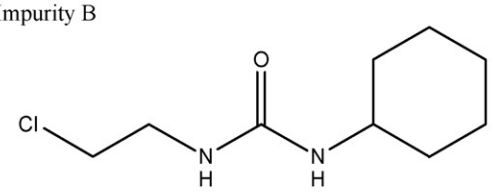
N-(2-chloroethyl)-*N*′-cyclohexyl-*N*-nitrosourea

(b) Impurity A



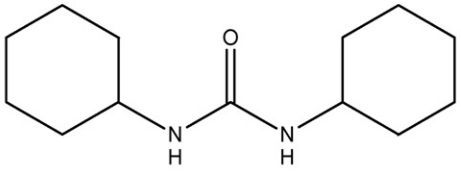
1,3-bis(2-chloroethyl)urea

(c) Impurity B



1-(2-chloroethyl)-3-cyclohexylurea

(d) Impurity C



1,3-dicyclohexylurea

Fig. 1. Structures of Lomustine and impurities. (a) Lomustine. (b) Impurity A. (c) Impurity B. (d) Impurity C.

trile (24:76, *v*/*v*). The mobile phase was filtered through a 0.45-μm nylon membrane. The mobile phase flow rate was 1.0 mL/min. The LC gradient was time (min)/%B: 0/25, 15/60, 32/70, 40/70, 42/25 and 50/25. The column was maintained at 40 °C and the spectrum was monitored at 205 nm. The injection volume was 20 μL. Acetonitrile was used as diluent during the preparation of the standard and test samples.

2.4. Preparation of standard solutions

A stock solution of Lomustine (5000 μg/mL) was prepared by dissolving an appropriate amount in the diluent. A stock solution of impurities (a mixture of Imp-A, Imp-B and Imp-C) at 500 μg/mL was also prepared in diluent.

2.5. Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. Degradation conditions employed were UV light (200 Wh/m^2), Visible light(1.2 million lux hours), thermal exposure to 60 °C, acid hydrolysis with 0.5N HCl, base hydrolysis with 0.1N NaOH, water hydrolysis and oxidative degradation using 3% H_2O_2. Peak purity testing was carried out on the stressed samples of Lomustine by using the PDA detector.

2.6. Method validation

2.6.1. Precision

The precision of the determination of the impurities was checked by injecting six individual preparations of (5000 μg/mL) Lomustine spiked with 0.10% of Imp-A, Imp-B and Imp-C and calculating the % RSD of area for each compound. The intermediate precision of the method was also evaluated using different analysts and a different instrument in the same laboratory.

2.6.2. Limit of detection and limit of quantification

The LOD and LOQ for Lomustine, Imp-A, Imp-B and Imp-C were estimated at a S/N of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations. Precision study was also carried at the LOQ level by injecting six individual preparations of Lomustine, Imp-A, Imp-B and Imp-C and calculated the %RSD for the areas.

2.6.3. Linearity

Linearity test solutions for the impurities were prepared by diluting the impurity stock solution to the required concentrations. The solutions were prepared from LOQ to 150% with respect to the impurities specification level of 0.20% (i.e. LOQ, 2.5 μg/mL, 5.0 μg/mL, 7.5 μg/mL, 10.0 μg/mL, 12.5 μg/mL and 15.0 μg/mL). The calibration curves were drawn by plotting the peak areas of impurities against the corresponding concentration. The slope and *Y*-intercept of the calibration curve was calculated.

2.6.4. Accuracy

Accuracy determination of the impurities was carried out in triplicate at 5 μg/mL, 10.0 μg/mL and 15.0 μg/mL of the Lomustine concentration (5000 μg/mL). The percentage recoveries for the impurities were calculated.

2.6.5. Robustness

To determine robustness, experimental conditions were purposely altered and the resolution of the impurities and analyte was evaluated. Flow rate was changed by 0.2 units, pH of buffer from 2.8 to 3.2 and column temperature was studied at 35 °C and 45 °C. In all the above conditions, the components of the mobile phase were held constant.

2.6.6. Solution stability and mobile phase stability

The solution and mobile phase stability of Lomustine and its impurities was carried out by a leaving spiked sample solution in a tightly capped volumetric flask at room temperature for 48 h. Content of impurities were determined at 24 h intervals.

3. Results and discussion

3.1. Optimization of chromatographic conditions

The main difficulty of the chromatographic method was to get the separation of Imp-C from the Lomustine peak. Attempts were

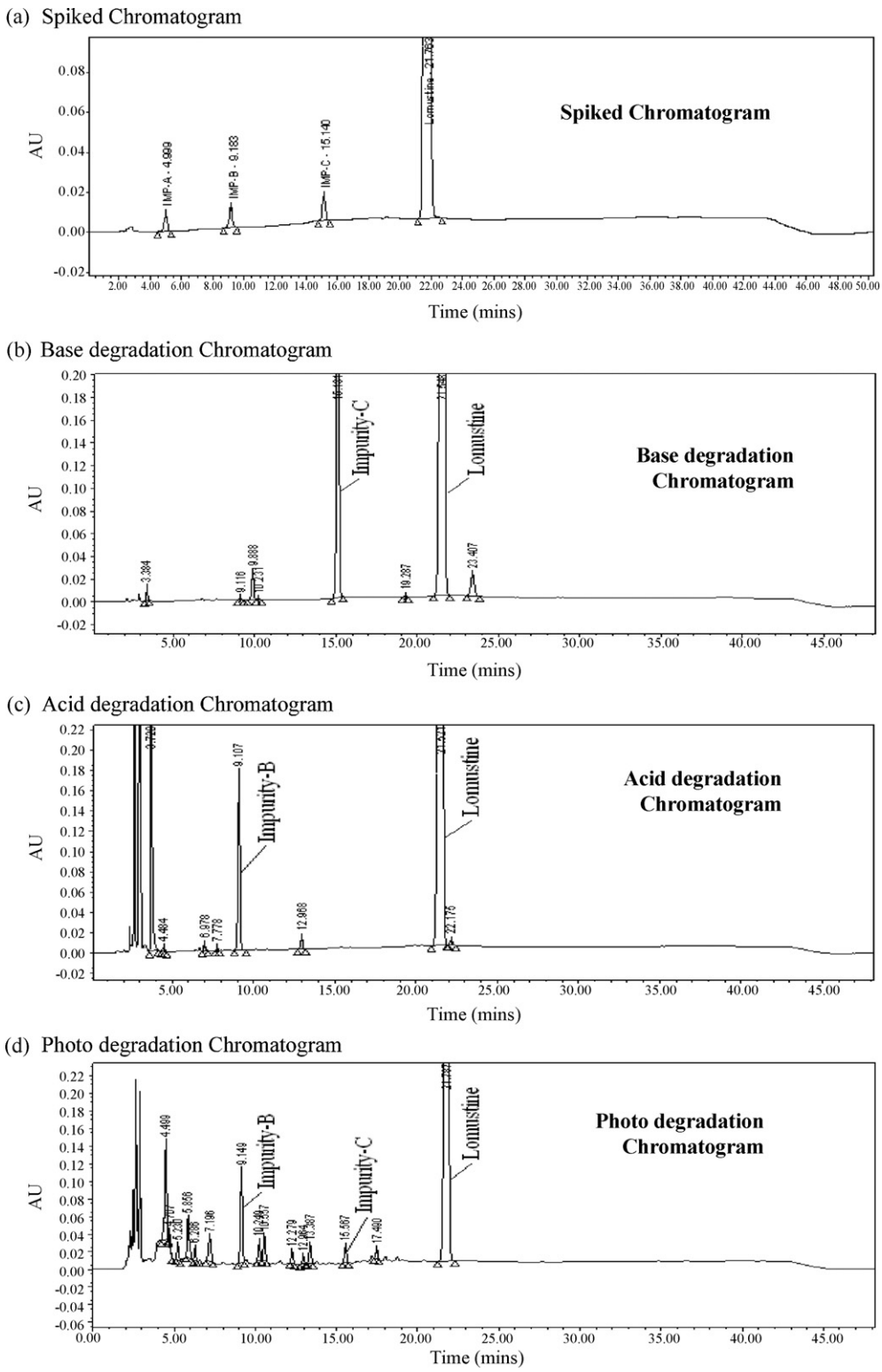


Fig. 2. Typical LC chromatograms of spiked sample, base, acid and photodegradation. (a) Spiked chromatogram. (b) Base degradation chromatogram. (c) Acid degradation chromatogram. (d) Photodegradation chromatogram.

made by using different C18 and C8 stationary phases [6]. Effects of pH (2–5) and ionic strength (1–10 mM) were investigated using phosphate and acetate buffers. It was found that the retention time of Lomustine did not significantly alter at pH 2–5 and ionic strength between 1 mM and 10 mM. But the change in the organic composition affects the separation of Imp-C from Lomustine. So the organic composition of mobile phase is critical on the separation of Imp-C from Lomustine. The optimum conditions are given in Section 2.

3.2. Results of forced degradation studies

Degradation was not observed in a Lomustine bulk sample during thermal exposure to 60 °C. Imp-B is the major degradent in acid,

oxidative and photodegradation. Imp-C is the major degradent in base degradation (Fig. 2). Peak purity test results confirmed that the Lomustine peak was homogeneous and pure in all the analyzed stress samples.

3.3. Results of method validation studies

3.3.1. Precision

The RSD of the area of Lomustine, Imp-A, Imp-B and Imp-C was within 2.2%. The RSD of results obtained in intermediate precision studies was within 0.7%.

3.3.2. Limit of detection and limit of quantification

The limits of detection (LOD) of Lomustine, Imp-A, Imp-B and Imp-C were found to be in the range of 0.15–0.20 μg/mL (of analyte concentration 5000 μg/mL). The limits of quantification (LOQ) of Lomustine, Imp-A, Imp-B and Imp-C were found to be in the range of 0.60–0.75 μg/mL. The precision for Lomustine, Imp-A, Imp-B and Imp-C at LOQ level was below 3.0% RSD.

3.3.3. Linearity

Linear calibration plots for the impurities and analyte were obtained over the calibration ranges tested, i.e. LOQ to 0.3% for Lomustine, Imp-A, Imp-B and Imp-C. The correlation coefficients obtained were greater than 0.999.

3.3.4. Accuracy

The percentage recovery of Imp-A, Imp-B and Imp-C in bulk drug samples ranged from 99 to 107%. Chromatograms of blank and spiked samples at 0.2% level of all three impurities in a Lomustine bulk drug sample are shown in Fig. 2.

3.3.5. Robustness

In all the deliberately varied chromatographic conditions (flow rate, pH and column temperature), the resolution between impurities and analyte was found to be more than 2.0.

3.3.6. Solution stability and mobile phase stability

The RSD of the three impurities during solution stability and mobile phase stability experiments was within 1.0%. No significant change was observed in the content of impurities during solution stability and mobile phase stability experiments confirm that sample solutions and mobile phase used during the study were stable up to 48 h.

4. Conclusions

The simple RP-LC method developed for the quantitative determination of related compounds of Lomustine and its possible degradation products is precise, accurate and specific for the analysis of bulk material. The method was fully validated, showing satisfactory results for all the parameters tested. The developed method is stability indicating and can be used for the routine analysis of production samples.

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