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Research Article

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A VALIDATED HPLC–UV METHOD FOR THE ANALYSIS OF METHYLISOCYANATE USING DIISOPROPYLAMINE AS A DERIVATIZING AGENT

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ABSTRACT

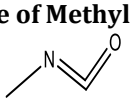
A simple and sensitive reversed-phase liquid chromatographic method has been developed for the determination of methyl isocyanate by derivatization. The method is based on the pre-column derivatization of methyl isocyanate with di-isopropyl amine. The derivatization reaction proceeds in aqueous solution at room temperature**.** The formation of the corresponding derivative of methyl isocyanate is instantaneous. A reversed-phase Xtera C-8 column (150 mm × 4.6 mm i.d., particle size 3.5 µ) with mobile phase consisting of 0.1% TFA and Methanol 60:40 (v/v) was used. The instrument was set at a flow rate of 1.0 mLmin^{-1}, at ambient temperature, and the wavelength of UV-visible detector at 216 nm. The method was suitably validated with respect to specificity, linearity of response, precision, stability in analytical solution, and robustness for its intended use. The method showed excellent linearity over a range of 504 µgmL^{-1} to 1080 µgmL^{-1}. The correlation coefficient was noted to be 0.9995.

Keywords: RP-HPLC; methyl isocyanate; derivatization; precolumn derivatization; diisopropylamine.

INTRODUCTION

Methyl isocyanate (MIC) chemically known as isocyanatomethane is a colorless highly flammable liquid that evaporates quickly when exposed to the air. It has a sharp and strong odor. MIC is used in the production of pesticides, polyurethane foam, and plastics. It is a severe pulmonary irritant and is extremely toxic to humans from acute (short term) exposure.[1-3] The structure of MIC is shown in Figure **1**.

Figure 1. The structure of Methyl isocyanate (MIC)



High-performance liquid chromatography (HPLC) method is one of the most popular methods of quantifying for chemical analysis. Methyl isocyanate has no significant UV absorption or fluorescence and thus derivatization by chromophoric reagents is inevitable for the sensitive detection of methyl isocyanate by HPLC with UV or fluorescence detectors.

Numerous methods based on derivatising agents have been reported for MIC.[4-21] One method contained only analytical conditions for MIC derivatized with Nitro Reagent (nitrobenzyl-N-n-propylamine) but no data were presented to indicate the collection efficiency of the sampling device. Another method was a failure report by NIOSH that had derivatized MIC with Nitro Reagent or NMA (naphthylmethylamine) in a toluene impinger. Since NIOSH had reported that the reaction time for derivatization was too slow in the impinger and that the derivatization reagents were unstable, NIOSH concluded that the procedure could not be validated. Union Carbide used a tube packed with a specially treated XAD-2 resin in series with a $CuCl_2$ (cupric chloride) bubbler. The tube was desorbed with a fluorescamine (Fluram) solution and analyzed by HPLC with a fluorescence detector but the procedure was not validated.

Considering above difficulties in analysis of MIC which is a key raw material for many drug substances, we developed a novel HPLC method for analysis of MIC. In present study methyl isocyanate response is measured by direct UV detection with enhanced sensitivity and method is simpler, highly reproducible, specific and accurate, compare to using complex techniques like titrimetry, spectrophotometric, capillary electrophoresis or GC technique. As methyl isocyanate does not contain any chromophoric group, it is very difficult to determine methyl isocyanate by direct UV detection. Hence a method has been optimized and developed by derivatizing methyl isocyanate with di-isopropyl amine. The method has been optimized with respect to derivatization temperature and suitably validate for its intended use.

MATERIALS AND METHODS

Chemicals and Reagents

Methanol and acetonitrile (HPLC-grade) was procured from SDFCL, S. D. Fine Limited, Mumbai, India. Ammonium acetate AR, di hydrogen potassium phosphate AR and ortho phosphoric acid AR grade were procured from S. D. Fine Limited, Mumbai, India. HPLC grade water was used throughout the study. Other chemicals used were of analytical or HPLC grade.

Instrumentation

The analysis was carried out on a Waters Alliance e-2695 separating module (Waters Co., MA, USA) using photo

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diode array detector (waters 2998) with auto sampler and column oven. The instrument was controlled by the use of Empower software (version 6.00.00.00) installed with equipment for data collection and acquisition.

Chromatographic Conditions

The separation was performed on Xtera C-8 column (150 mm × 4.6 mm i.d., particle size 3.5 µ) with mobile phase at the flow rate of 1.0 $mLmin^{-1}$ was used. The mobile phase consisted of 0.1% TFA and Methanol 60:40 (v/v) filtered through 0.45-µm nylon filter, and degassed in ultrasonic bath prior to use. Measurements were made with injection volume 10 µL and UV detection at 216 nm. All analyses were performed at ambient temperature.

Derivatization process of standard and sample solution

Standard solution preparation: Accurately weighed 28 mg of 1,1-diisopropyl-3-methylurea (100 mg 1,1-diisopropyl-3-methylurea equivalent to 36 mg of MIC) was transferred into a 100 mL volumetric flask, and dissolved in the acetonitrile. The solution was shaken at room temperature and volume was made upto the mark with acetonitrile. The working standard solution was prepared by diluting the stock standard solution with acetonitrile to get the concentration of 2 μgmL^{-1}.

Sample solution preparation: A sample solution was prepared by dissolving 2 mL of diisopropyl amine with 10 mL of acetonitrile in 25 mL volumetric flask. Accurately weighed 50 mg of drug substance was added. The solution was shaken at room temperature and volume was made upto the mark with acetonitrile. The final concentration was 0.8 $mgmL^{-1}$.

Method Validation

The optimized chromatographic conditions were validated by evaluating specificity, range, linearity, precision, robustness and system suitability parameters in accordance with the ICH guidelines Q2 (R1).[22-24]

Range and Linearity: The range of an analytical method is the interval between the upper and lower levels of the analytes for which the method shows adequate precision, accuracy and linearity. A desired concentration range is often known before starting the validation of the method. Linearity is studied to determine the range over which analyte response is a linear function of concentration. This study was performed by preparing standard solutions at seven different concentrations. The solutions were injected in triplicate and curve was constructed. The responses were measured as peak area. The calibration curve was obtained by plotting peak area against concentration. Slope, intercept and coefficient of variation (R^2) of the calibration curve were calculated to assess linearity of the method.

Specificity: Specificity is the ability of the method to accurately measure the analyte response in the presence of all potential components. The method specificity was assessed by comparing the chromatograms (HPLC) obtained from the blank, standard solution and sample solution of MIC derivative.

Precision: The precision of an analytical method is the closeness of replicate results obtained from analysis of the same homogeneous samples. Precision was considered at two levels, i.e. repeatability and intermediate precision, in accordance with ICH recommendations. Results from determination of repeatability and intermediate precision were expressed as SD and RSD.

Robustness: The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness of the method was investigated under a variety of conditions including flow rate and percentage of methanol in the mobile phase.

Solution Stability: To assess the solution stability, standard and sample solutions were kept at 25±2°C (laboratory temperature) for 24 h. These solutions were compared with freshly prepared standard and test solutions.

System Suitability Testing: System suitability tests are integral part of gas and liquid chromatography methods. They are used to verify adequacy of the resolution and reproducibility of system. For system suitability parameters, five replicate injections of standard solution were injected and parameters such as the tailing factor, theoretical plate, retention volume and % RSD of the peak were calculated.

RESULTS AND DISCUSSION

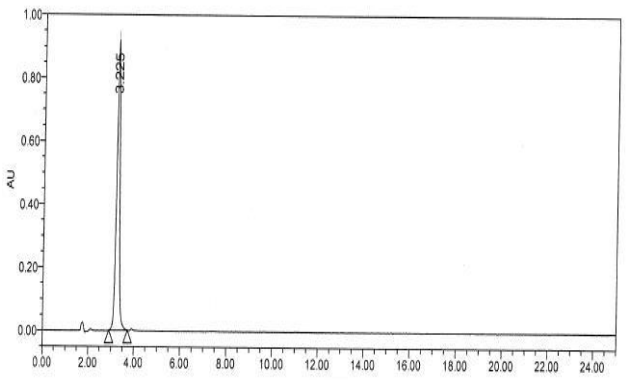
In this work an analytical LC method for the determination of methyl isocyanate by derivatization with UV detection was developed and validated as per ICH guidelines for analytical method validation, Q2 (R1).

Method Development

The chromatographic method was developed and validated by assessing linearity, selectivity, precision and robustness. The focus was on the selection of mobile phase and its composition as well as other parameters like flow rate and working wave length. Several mobile phase compositions were tried. During the optimization of the method, two columns (Xtera C-8 column (150 mm × 4.6 mm i.d., particle size 3.5 µ) and Zorbax XDB, C-18, 15cm×4.6mm, particle size 3.5 µ) and two organic solvents (acetonitrile and methanol) were used. The chromatographic conditions were also optimized by using different buffers in mobile phase preparation. After a series of screening experiments, it was concluded that 0.1%TFA gave better peak shapes.

Finally, the mobile phase containing 0.1% TFA and Methanol 60:40 (v/v) was selected because it resolved the peaks of methyl isocyanate derivative efficiently. A typical HPLC chromatogram obtained is given in Figure **2**. Quantification was done on the basis of peak area at 1.0 $mLmin^{-1}$ flow rate with UV detection at 216 nm at room temperature.

Figure 2. HPLC chromatogram of standard solution



Method Validation

An optimized method must be validated before actual use. System suitability testing was performed as per ICH guidelines for analytical method validation, Q2 (R1). The

validation studies were performed as follows.

Specificity: Blank, standard solution and sample solution of MIC derivative were chromatographed individually as per the method to examine interference, if any, with MIC derivative peak. No peak from the blank was observed at the retention time of MIC derivative peak. (Figure **3**, **4**, **5**).

Figure 3. HPLC chromatogram of blank

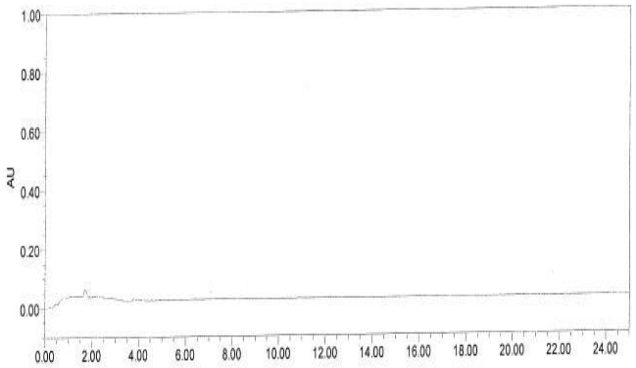


Figure 4. Peak purity plot of standard solution

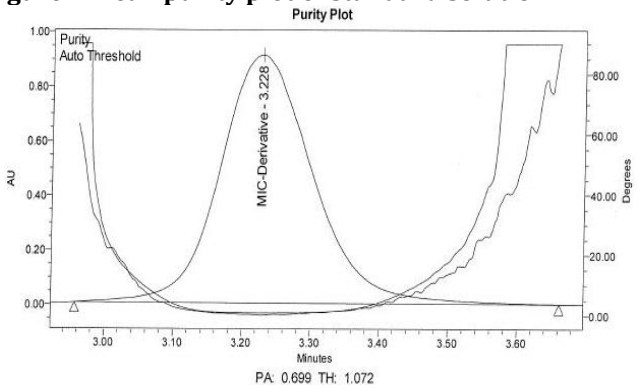
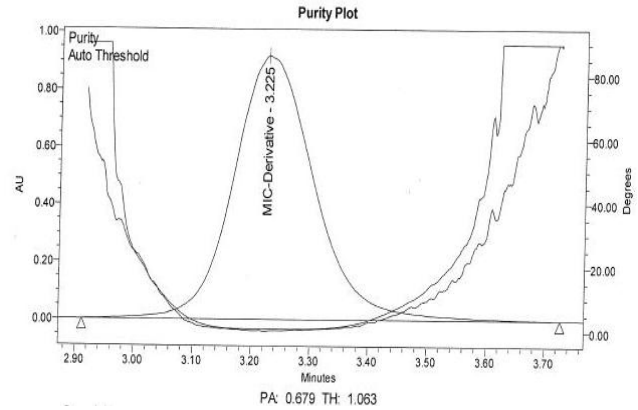
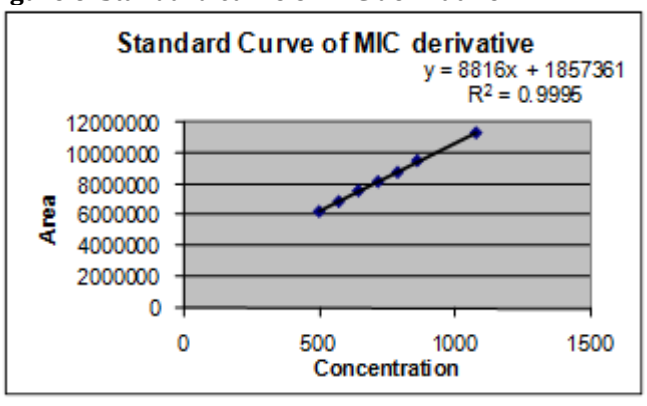


Figure 5. Peak purity plot of sample solution



Range and Linearity: Seven different concentrations (504, 576, 648, 720, 792, 864 and 1080 µgmL^{-1}) of the standard solution were prepared for linearity studies. The calibration curve obtained by plotting peak area against concentration showed linear relationship. Calibration curve is shown in Figure **6**.

Figure 6. Standard curve of MIC derivative



The linear regression equation for MIC derivative was found to be y = 8816x + 1857361. The regression coefficient (R^2) value for MIC derivative was noted 0.9995. The results showed that an excellent correlation existed between peak area and concentration of drug within the selected concentration range. The results confirmed the linearity and the reproducibility of the assay method. Regression characteristics of the proposed HPLC method are given in Table **1**.

Table 1. Linearity parameters for the MIC derivative

Linearity parameter	MIC derivative
Range (µgmL^{-1})	504 µgmL^{-1} to 1080 µgmL^{-1}
Slope	8816
Intercept	1857361
Regression coefficient (R^2)	0.9995

Precision

System precision: Five injections of standard solution were injected into the HPLC system. Data shown in Table **2** indicate an acceptable level of precision for the analytical system. (Acceptance criteria: RSD should not be >2.0%).

Table 2. System precision

Injection No.	Area
1	8340626
2	8357995
3	8394868
4	8352997
5	8355270
Mean	8360351
SD	20408
% RSD	0.24

Method precision: Five injections of sample solution were analysed by the proposed method. Data is shown in Table **3**. The % RSD value indicates that the method has an acceptable level of precision (Acceptance criteria: RSD should not be > 2.0%).

Table 3. Method precision

Injection No.	Area
1	8128705
2	8181200
3	8115560
4	8122852
5	8135606
Mean	8136784
SD	25903
% RSD	0.32

Robustness: The robustness of a method is the ability to remain unaffected by small changes in parameters. The experimental conditions were purposely altered. To study the effect of organic solvent (methanol) composition on asymmetry, the concentration was changed 2 units on either side from 40 to 38 and 42, while other chromatographic conditions were held constant. To study the effect of flow rate on asymmetry, it was changed 0.1 unit on either side from 1.0 to 1.1 mLmin^{-1} and 0.9 mL min^{-1}, while other conditions were held constant. The robustness results are shown in Table **4**.

Table 4. Data for robustness study

Parameter altered	Asymmetry (*As*)	RSD (%)
Increased organic solvent (58:42)	1.39	1.04
Decreased organic solvent (62:38)	1.31	0.29
Increased flow rate (1.1 mLmin^{-1})	1.41	0.41
Decreased flow rate (0.9 mLmin^{-1})	1.46	0.37

Stability: To confirm the stability of standard and sample solutions during the analytical process, both the solutions were analyzed over a period of 24 h at 25 °C (laboratory temperature). The results showed that both the retention time and the peak area of the drugs were unchanged

(%RSD less than 0.96%) and no significant degradation was observed within the period sufficient for performing analytical process.

System Suitability Parameters

The system suitability test ensures the validity of the analytical procedure as well as confirms the resolution between different peaks of interest. For system suitability parameters, five replicates of standard solution were injected. All critical parameters met the acceptance criteria on all days. Parameters such as tailing factor, theoretical plate, retention volume and % RSD of the peak was calculated. The results are shown in Table **5**.

Table 5. System suitability data

Parameters	MIC derivative
Retention time (min)	3.22
Theoretical plates	2855
Tailing factor	1.18
Retention volume	3.22
% RSD	0.24

CONCLUSION

A simple isocratic reverse phase method for the determination of methyl isocyanate was developed and validated using direct UV detection. The method is selective, precise and accurate. Methyl isocyanate is not easily detected by HPLC using UV detection because of absence of a chromophoric group. Derivatization with diisopropyl amine is a simple and very effective means of enhancing the chromatographic detection of the compound. The proposed method was also found to be robust with respect to flow rate and composition of mobile phase. In addition, simple isocratic elution and easy extraction procedure offered rapid and cost-effective analysis.

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