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A New Gradient RP- HPLC Method for Quantitative Analysis of : (3-Fluoro-4-Morpholin-4-yl-Phenyl)-Carbamic Acid Methyl Ester and its Related Substances

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ABSTRACT:

The present research describes a validated and novel RP-HPLC method for the quantitative analysis of (3-fluoro-4-morpholin-4-yl-phenyl)-carbamic acid methyl ester and its two related substances in KSM (Key Starting material). The chromatographic separation was achieved on a C18 stationary phase with a gradient mobile phase prepared from methanol and phosphate buffer. The quantification was carried out by UV detection at 250 nm. The developed RP-LC method was validated according to ICH guidelines. The percent recovery for individual substances at 25, 50, 100 and 150% of specification concentrations were found to be between 95 to 105% Indicating the accuracy of the method. The %RSD for system precision was found to be less than 2.0. The %R.S.D for repeatability and intermediate precision for the process-related impurities in (3-fluoro-4-morpholin-4-yl-phenyl)-carbamic acid methyl ester were found to be less than 1.0. The correlation coefficient of (3-fluoro-4-morpholin-4-yl-phenyl)-carbamic acid methyl ester and its two related substances was found to be greater than 0.99. The developed LC method can be used for the quantitative determination of (3-fluoro-4-morpholin-4-yl-phenyl)-carbamic acid methyl ester and its two related substances in KSM.

KEY WORDS: (3-fluoro-4-morpholin-4-yl-phenyl)-carbamic acid methyl ester, ICH guidelines, Validation, RP-HPLC, KSM

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INTRODUCTION:

(3-fluoro-4-morpholin-4-yl-phenyl)-carbamic acid methyl ester is KSM (Key Starting material) is used to synthesis the Linezolid API in one of route of synthesis.

The Route of synthesis of (3-fluoro-4-morpholin-4-yl-phenyl)-carbamic acid methyl ester is as below.

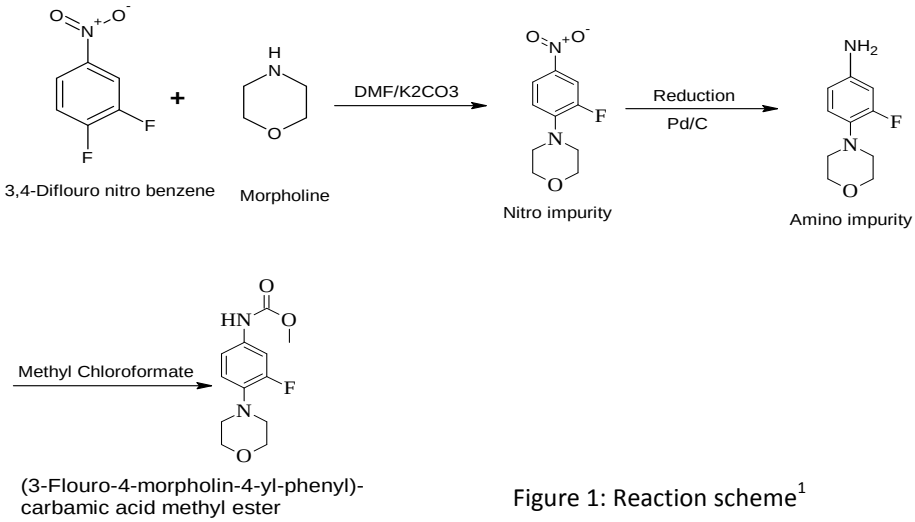

Figure 1: Reaction scheme[1]

During the manufacturing of (3-fluoro-4-morpholin-4-yl-phenyl)-carbamic acid methyl ester little amount of impurities are always carried in to final Product. Two possible impurity identify for method development. Name of these two impurities are Nitro impurity and Amino impurity.

Both impurities Structure are in above reaction scheme. However there is no reported LC method by an exhaustive computer- Assisted literature survey method was validated according to ICH guidelines. Here we describe the investigation in detail.

The Liquid Chromatographic system consisted of quaternary gradient pump, 25°C, column oven and a UV detector. The output signal was monitored and integrated using LC solutions chromatography manager software (Prominence HPLC, Shimadzu, Japan).

MATERIALS AND METHODS

Chemicals and Reagents

Samples of (3-fluoro-4-morpholin-4-yl-phenyl)-carbamic acid methyl ester and its related impurities Nitro impurity and Amino impurity were procured from Venkar Chemicals Private Limited Hyderabad . HPLC grade Methanol and analytical Potassium dihydragen Phosphate was obtained from Merck .(Mumbai, India). High purity water was prepared by Millipore Milli-Q plus purification system (Millipore, Bedford, USA).

Equipment

Chromatographic conditions

The chromatographic column used was ProntoSil 120-5-C18-ace-EPS, 250 x 4.6 mm, 5µm particle. Mobile phase consisted of A and B. Mobile phase A was 20 Mm potassium dihydrogen. This solution was filtered through 0.45 µm nylon filter. Mobile phase B used was HPLC grade Methanol. Theflow rate of the mobile phase was as per Gradient program. The gradient program applied is shown in Table 1. The sample injection volumewas 20 µL and the quantification were achieved by UV detector at 250nm.

Diluent

A mixture of mobile phase A and methanol in the ratio of 50:50 V/V was used as diluent. The above solution was filtered through a 0.45µ nylon membrane filter prior to use.

Preparation of test solution: About 25.0mg of (3-fluoro-4-morpholin-4-yl-phenyl)-carbamic acid methyl ester sample was weighed accurately and transferred into 50 mL standard volumetric flask. It was dissolved in sufficient quantity of diluent and made up to the mark with the same diluent. Preparation of impurity mixture.

About 5.0mg of each (3-fluoro-4-morpholin-4-yl-phenyl)-carbamic acid methyl ester related impurities viz: Nitro impurity and Amino impurity and (3-fluoro-4-morpholin-4-yl-phenyl)-carbamic acid were weighed accurately and transferred to 10 mL standard volumetric flask. and dissolved in diluent and made up to the mark. 1.0 mL of this solution was diluted to 100mL with diluent.1.0mL of this solution further diluted to 10 mL with diluent. (i.e. 0.1% of each impurity with respect to (3-fluoro-4-morpholin-4-yl-phenyl)-carbamic acid methyl ester at the concentration of 0.5mg/mL and injected in to the system.

Method Validation[2-8]

Specificity study

The specificity of the developed LC method for related substances of (3-fluoro-4-morpholin-4-yl-phenyl)-carbamic acid methyl ester was evaluated by Injecting (3-fluoro-4-morpholin-4-yl-phenyl)-carbamic acid methyl ester , Amino impurity and Nitro Impurity separately for retention time confirmation. Retention time table-1 is as below.

Table -1: Retention time table

Name	Retention Time
Amino impurity	About 7.2 min
Nitro impurity	About 19.3 min
(3-fluoro-4-morpholin-4-yl-phenyl)-carbamic acid methyl ester	About 13.5 min

Linearity

Standard solutions at nine different concentration levels ranging from LOQ to 0.15µg/mL (150% of specification limit) were prepared and analyzed in duplicate to demonstrate the linearity for

(3-fluoro-4-morpholin-4-yl-phenyl)-carbamic acid methyl ester and two four impurities. The calibration curves were plotted for two impurities using area counts versus corresponding concentrations. The slope, Y-intercept and correlation coefficient were calculated. The response factors were

calculated by comparing the slope of the calibration curve for the impurities (Amino impurity and Nitro impurity) with that of the (3-fluoro-4-morpholin-4-yl-phenyl)-carbamic acid methyl ester The related substances were quantified against area count of respective impurity in standard solution.

Accuracy

Accuracy of the method was demonstrated by using (3-fluoro-4-morpholin-4-yl-phenyl)-carbamic acid methyl ester spiked with a related impurities at four different concentration levels in triplicate. The analyses were carried out at 25%, 50%, 100% and 150% of specification limit as per ICH guidelines. The mean recoveries of all the impurities were calculated.

Precision

System precision of the method was evaluated by injecting (3-fluoro-4-morpholin-4-yl-phenyl)-carbamic acid methyl ester standard solution six times and calculated the percent relative standard deviation (%RSD) for area of (3-fluoro-4-morpholin-4-yl-phenyl)-carbamic acid methyl ester peak. The precision of the method for the determination of impurities related to (3-fluoro-4-morpholin-4-yl-phenyl)-carbamic acid methyl ester, was studied for repeatability and intermediate precision. Repeatability was demonstrated by analyzing the synthetic homogeneous solution containing 0.1 % of each impurity spiked to (3-fluoro-4-morpholin-4-yl-phenyl) carbamic acid methyl ester sample for six times. The %RSD for peak area of each impurity was calculated. Intermediate precision of the method was demonstrated by analyzing same sample of (3-fluoro-4-morpholin-4-yl-phenyl)-carbamic acid methyl ester on three different days (inter day) and intra-day variations of impurities of (3-fluoro-4-morpholin-4-yl-phenyl)-carbamic acid methyl ester are expressed in terms of %R.S.D values.

Limit of detection (LOD) and Limit of quantitation (LOQ)

The limit of detection and limit of quantitation for (3-fluoro-4-morpholin-4-yl-phenyl)-carbamic acid methyl ester , Amino impurity and Nitro impurity were calculated from the linearity data using residual standard deviation of the response and slope of the calibration curve for each impurity

Precision LOQ level

Precision of the method was also evaluated by injecting standard solutions of Amino impurity and Nitro impurity at about the predicted LOQ levels for six times separately and calculated the percent relative standard deviations (%RSD) for area of each impurity peak.

Robustness

In order to demonstrate the robustness of the method, system suitability parameters were verified by making deliberate changes in the chromatographic conditions, viz. change in flow rate by ±0.1 mL/ min, and change in the column temperature by • ±3°C, keeping the rest of the chromatographic conditions for each alteration study constant.

RESULTS AND DISCUSSION

Initial separation experiments

The main aim of the chromatographic method is to separate (3-fluoro-4-morpholin-4-yl-phenyl)-carbamic acid methyl ester and its two related impurities in the product. Different reverse phase stationary phase were employed during method development and different kind of mobile phase with different pH condition were studied with combination of Methanol. The resolution The gradient program was optimized in order to elute both impurities and (3-fluoro-4-morpholin-4-yl-phenyl)-carbamic acid methyl ester. chromatographic separation of all the impurities was achieved successfully by following the gradient program tabulated in Table -2 using the mobile phase as mentioned under the experimental section.

Table 2: **Gradient programme:**

Time in min	Flow rate (mL/min)	Mobile phase-A (%)	Mobile phase-B (%)
0.0	1.00	75	25
6.0	1.00	75	25
20.0	1.00	25	75
21.0	1.00	75	25
25.0	1.00	75	25

The typical retention times of (3-fluoro-4-morpholin-4-yl-phenyl)-carbamic acid methyl ester, Amino impurity and Nitro Impurity are about 7.3, 14.1 and 19.6 min,

respectively. Standard chromatogram of (3-fluoro-4-morpholin-4-yl-phenyl)-carbamic acid methyl ester , Amino impurity and Nitro Impurity (0.5µg/mL) is shown in Fig.2. A typical chromatogram of (3-fluoro-4-morpholin-4-yl-phenyl)-carbamic acid methyl ester sample is also shown in (Fig. 3). It is clear that all the compounds were eluted and separated with good peak shapes and resolution.

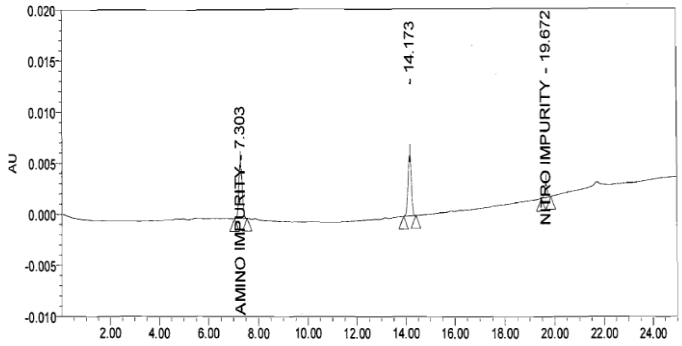

Figure. 2: HPLC Chromatogram showing (0.5µg/mL) of standard solution of (3-fluoro-4-morpholin-4-yl-phenyl)-carbamic acid methyl ester, Amino impurity and Nitro Impurity

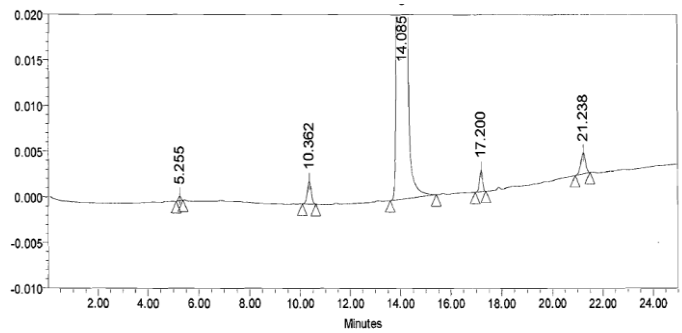

Figure 3 HPLC Chromatogram showing (0.5mg/mL) of standard solution of (3-fluoro-4-morpholin-4-yl-phenyl)-carbamic acid methyl ester

Method Validation

Specificity study

Table -1 clearing indicating that the method is specific and able to resolve all the process-related impurities.

Linearity

The linearity regression analysis was demonstrated to check the acceptability of the method for quantitative determination range of LOQ to 150% of the specification limit. The coefficient of correlation was found to be more than 0.9997. The values of slope, intercept, correlation coefficient, LOD and LOQ for each impurity were shown in Table 3.

Table 3: Summary of linearity, LOD and LOQ

Substance	Correlation coefficient	LOD (µg/mL)	LOQ (µg/mL)
Amino Impurity	0.9997	0.009	0.021
Nitro Impurity	0.9995	0.033	0.05

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD values were found to be 0.009, 0.033, µg/mL for impurities Amino impurity and Nitro impurity respectively. LOQ values were found to be 0.021, 0.05 µg/mL for Amino impurity and Nitro impurity respectively (Table 3)

Table 4: Precision studies for impurities at LOQ level.

Substance	LOQ level Mean peak area	%RSD (n=6)
	Mean* peak area	
Amino Impurity	1073	3.6
Nitro Impurity	510	4.6

* Average of six determinations

Precision at LOQ level

Precision of the method was also checked at about predicted level of LOQ. The %R.S.Ds for each impurity was shown in Table 4. Precision

Accuracy

The percent recovery for individual substances at 25, 50, 100 and 150% of specification concentrations for Amino impurity and 50, 100 and 150% of specification concentrations for Nitro impurity. (Table 5) were found to be between 95 to 105% indicating the accuracy of the method.

Table 5: Parameters of recovery of impurities of (3-fluoro-4-morpholin-4-yl-phenyl)-carbamic acid methyl ester

Name	Spike level	% Recovery *
Amino Impurity	25	91
Amino Impurity	50	96
Amino Impurity	100	102
Amino Impurity	150	104
Nitro Impurity	50	93
Nitro Impurity	100	98
Nitro Impurity	150	101

* Average of three determinations

Table 6: Precision studies for (3-fluoro-4-morpholin-4-yl-phenyl)-carbamic acid methyl ester and its impurities

Substance	Method precision %RSD(n=6)	Intermediate precision	Inter day %RSD(n=6)
		Intraday %RSD(n=6)	
Amino Impurity	0.56	0.43	0.52
Nitro Impurity	0.83	0.73	0.87

Precision

The %RSD for system precision was found to be less than 2.0. The precision of the method for the determination of impurities related to (3-fluoro-4-morpholin-4-yl-phenyl)-carbamic acid methyl ester sample.Repeatability and intermediate precision for impurities in (3-fluoro-4-morpholin-4-yl-phenyl)-carbamic acid methyl ester were found to be less than 1.0 %R.S.D. The results are presented under Table 6, which confirm good precision of the method.

Robustness

The method was demonstrated to be robust over an acceptable working range of its HPLC operational conditions. The system suitability results within the acceptable limits and selectivity of individual substances were also not affected when subjected deliberately for varied chromatographic conditions. The result of the study confirms the robustness of the method.

CONCLUSION

The validated RP- LC method developed for the quantification of (3-fluoro-4-morpholin-4-yl-phenyl)-carbamic acid methyl ester and its related substances in Product was reported. The method was found to be selective, sensitive, precise and accurate. The developed RP- LC method showed satisfactory results for all tested method validation parameters . The formulated method can be used for assessing the impurities in the drug substance. The developed method can be conveniently used by quality control departments to determine the related substance and in regular production samples of this intermediate.

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REFERENCES:

1. Barbachyn M R, Brickner S J, Hutchinson D. Substituted oxazine and thiazine oxazolidinone antimicrobials K. Patent no. EP 0717738B1

2. ICH, Q2B (2005) Validation of Analytical Procedure: Methodology, International Conference on Harmonization, IFPMA, Geneva, Switzerland.

3. FDA, ICH-Q1A (R2): Stability Testing of New Drug Substances And Product, Vol. 68, U S Food and Drug Administration, Washington, DC, USA, 2nd edition 2003.

4. FDA, ICH-Q1B: Photo-stability Testing of New Drug Substance and Product, Vol. 62, U S

   Food and Drug Administration Washington, DC, USA, 1997.

5. FDA, ICH-Q2 (R1): Validation of analytical Procedures: Text and Methodology, Vol. 60, US Food and Drug Administration, Washington, DC, USA, 1995.

6. United State Pharmacopoeia 30 – National Formulary 25, The U.S. Pharmacopoeia Convention, United State