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Estimation of lactose in swab and air samples by using LCMS-MS

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ABSTRACT

The objective of the current study was to develop and verify LCMS method for the estimation of lactose in swab and air sample. The stationary phase was a Supelcosil, 3µm (50 x 4.6 mm i.d., 3µ) column. The mobile phase was prepared by mixing acetonitrile: 5mM ammonium formate (80:20, v/v). Detection was made at m/z 360.3 / 163.2 using ESI Positive ion spray ionization mode. The method was found to be linear in the concentration range of 3.089-51.490 ng/ml. The method was successfully applied for estimation of the lactose in swab and air sample. The concentration of samples was found to be below detection limit. A typical LCMS analysis is done in less time, resulting in a savings of more than a full day over HPLC methods. In addition to throughput rates, HPLC requires additional time for column equilibration and mobile phase preparation. Another significant factor in HPLC analysis is the handling and costs associated with the purchase and disposal of eluting solvents. LCMS eliminates all of these elements, saving both time and cost.

KEYWORDS

Lactose;
Cleaning validation;
LCMS.

INTRODUCTION

Cross contamination with active ingredients is a real concern. The Code of Federal Regulations (CFR) states that "Equipment and utensils shall be cleaned, maintained, and sanitized at appropriate intervals to prevent malfunctions or contamination that would alter the safety, identity, strength, quality, or purity of the drug product beyond the official, or other established requirements"[1]. Cleaning validation is required in the pharmaceutical field to avoid potential clinically significant synergistic interactions between pharmacologically active chemicals. Since the issuance of the US Food and Drug Administration's "Guide to Inspection of Validation of Cleaning Process" in July 1993 (3), cleaning validations have received increasing attention[2-8].

Cleaning validation tests the efficacy of cleaning methods used in pharmaceutical research and manufacturing. It is a critical and time intensive step. Process equipment must be cleaned after every stage in the drug development process and the equipment cannot be reused until the cleaning is validated. In cleaning validation, samples are taken from the equipment and analyzed for pre-determined thresholds of probable contaminants, in particular for active pharmaceutical ingredients (APIs) from previous batches[9-14].

Concern regarding the cleaning of pharmaceutical

processing equipment and operator exposure to active pharmaceutical ingredients (API) has been growing steadily recently, driven mainly by the increasingly strict regulations regarding operator safety and the occurrence of more potent active compounds[15-17].

The objective of this work is to present the development and verify LCMS method for the estimation of lactose in air and swab samples collected from the production premises for validating the cleaning, as well as to verify the method developed for lactose.

Study protocol

A study protocol was developed as per the guidelines and regulatory requirements; it defines the specific sampling locations within the production premises, analytical method and acceptance criteria.

Sample collection and identification

Swab and air samples

The location for swab samples are Pass box, Dispensing Chamber left side, Dispensing Chamber right corner, Dispensing Chamber right side, Drum Loading Chamber, Loading chamber, Operator, Floor, Air lock after activity. Refer TABLE 1.

The location for air samples is Chest, Exhaust. Refer TABLE 2

TABLE 1 : Swab samples collection location and identification

S. No	Sample Details				Location
	Run 01	Run 02	Run 03	Run 04	
1	Glove port (1.1)	Glove port (2.1)	Glove port (3.1)	Glove port (4.1)	Pass box
2	Glove port (1.2)	Glove port (2.2)	Glove port (3.2)	Glove port (4.2)	Dispensing Chamber left side
3	Gasket (1.3)	Gasket (2.3)	Gasket (3.3)	Gasket (4.3)	Dispensing Chamber right corner
4	Glove port (1.4)	Glove port (2.4)	Glove port (3.4)	Glove port (4.4)	Dispensing Chamber right side
5	Glove port (1.5)	Glove port (2.5)	Glove port (3.5)	Glove port (4.5)	Drum Loading Chamber
6	Gasket (1.6)	Gasket (2.6)	Gasket (3.6)	Gasket (4.6)	Loading chamber
7	Forehead of Operator (1.7)	Forehead of Operator (2.7)	Forehead of Operator (3.7)	Forehead of Operator (4.7)	Operator
8	Floor (1.8)	Floor (2.8)	Floor (3.8)	Floor (4.8)	Floor
9	Air lock (1.9)	Air lock (2.9)	Air lock (3.9)	Air lock (4.9)	Air lock after activity
Total	09	09	09	09	
Extra	Blank	Blank	Blank	Blank	

TABLE 2 : Air (Filter paper) samples collection location and identification

S.No	Run 01	Run 02	Run 03	Run 04	Location	Remarks
1	Air Sampler [P]*	Air Sampler [P]*	-	Air Sampler [P]*	Chest	* P- Air sample taken at personnel [filter paper]
2	-	-	Air Sampler [E]**	-	Exhaust	**E – Air sample taken at Exhaust [filter paper]
	1	1	1	1	Total	4

*** P- Air sample taken at personnel [filter paper]; **E – Air sample taken at Exhaust [filter paper]**

EXPERIMENTAL

Instrumentation

Ultra flow liquid chromatography Tandem Mass Spectrometry was used for sample analysis. Mass Spectrometry Model API 4000, UFLC model is UFLC XR equipped with a model LC-20ADXR a binary pump, SIL-20ACXR auto sampler used to keep temperature at 10°C, CTO-20AC column oven used to keep temperature at 30°C and CBM-20Alite system controller. Detection was made at m/z 360.3 / 163.2 using ESI Positive ion spray ionization mode. Analyst 1.5.1 software was used for the quantification. The stationary phase was a Supelcosil, 3µm (50 x 4.6 mm i.d., 3µ) column. The mobile phase was prepared by mixing acetonitrile: 5mM ammonium formate (80:20, v/v). The injection volume was 10 µl

and run time was 2 minutes. The mobile phase was filtered using a 0.45 μm membrane filter (Millipore) and sonicated with ultrasonicator. The mobile phase flow rate was 0.4 ml/min.

Chemicals and reagents

The reference standard of Lactose was provided by Strides Arco Lab (Bangalore, India). High purity water was prepared in-house using a Milli-Q water purification system obtained from Millipore (India) Pvt. Ltd. (Bangalore, India). HPLC grade methanol and acetonitrile and Ammonium formate.

Solution preparation

Stock solution

Weighed accurately 5.149 mg of lactose and dissolved in water and made up to 5 ml with methanol to get a concentration of 1029 μg/mL.

Buffer preparation (5 mM Ammonium formate)

Weighed accurately 155.01 mg of ammonium formate and dissolved in 500 ml of Milli-Q water and mixed well.

Mobile phase preparation (Buffer: Acetonitrile :: 20:80 v/v)

Around 200 mL of buffer and 800 mL of acetonitrile were added in 1000 mL measuring cylinder. Mixed well and sonicated.

Diluent solvent (Water: Acetonitrile :: 20:80 v/v)

Around 100 mL of water and 400 mL of acetonitrile were added in 500 mL measuring cylinder. Mixed well and sonicated.

Sample preparation and analysis

Swab samples

1 ml of sample from the swab was diluted to 10 ml with the dilution solvent and transferred into HPLC vials.

Air (Filter paper) samples

10 ml of dilution solvent added into petridish, soaked and shaken for a minute and transferred into HPLC vials.

The above samples were injected along with Linearity standards and QC samples which were interspersed throughout the batch.

System suitability

To verify that the analytical system is working properly and can give accurate and precise results, the system suitability parameters are to be set. Injected system suitability solution (6 injections of QCM) into LCMS and recorded the chromatograms. The RSD of analyte was 1.16%.

Limit of quantification

Limit of quantification is the lowest amount of analyte in a sample that can be detected and quantified under the stated experimental conditions.

To verify the LOQ, prepared 3.089 ng/mL solution of lactose and injected 6 replicates into LCMS and recorded the data. The RSD of analyte was 3.15 %.

Acceptance criteria

The LLQ for lactose from replicate standard preparation injections should not be more than 4.0 %.

Data processing

Acquired chromatograms using the computer based Analyst software version 1.5.1. Data was processed by peak area method. The concentration of the unknown is calculated from the following equation using regression analysis of spiked calibration standard with the reciprocal of the drug concentration using $1/x^2$.

$$y = mx + c$$

Where, y = peak area of analyte, m = slope of calibration curve, x = concentration of analyte, c = y-axis intercept of the calibration curve

LCMS method development and sample analysis

In this work, in order to validate the LCMS method for Lactose, the parameters of linearity, precision and accuracy were evaluated.

Linearity

In order to assess the linearity of the method, seven dilution of the lactose (3.089, 5.149, 7.714, 15.447, 25.745, 41.192, and 51.490 ng/ml) were used at LCMS method for the standard curves. The calculation of regression line was employed by the method of least squares.

Similarly 3 quality control samples were prepared as Low, Mid and High respectively at concentrations 7.698, 29.607 and 47.628 ng/ml (Figure 1, 2, 3).

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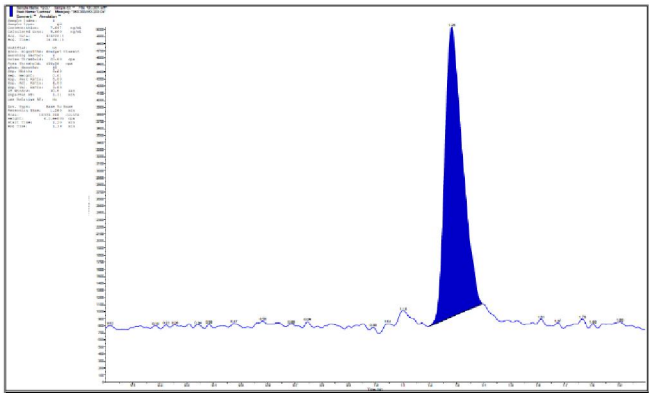

Figure 1 : QCL of lactose standard solution

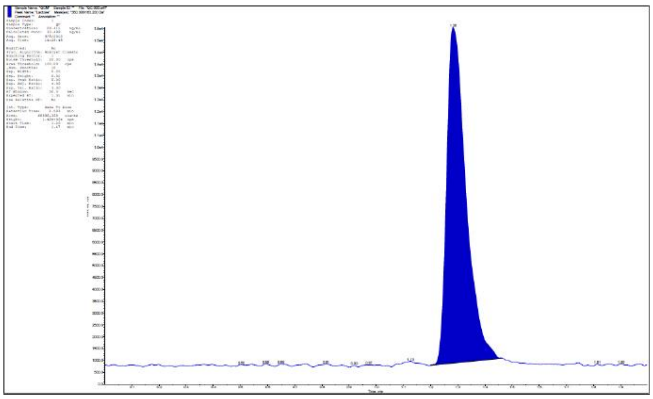

Figure 2 : QCM of lactose standard solution

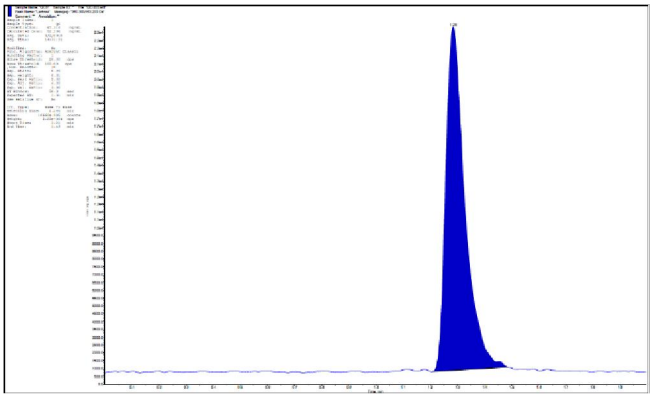

Figure 3 : QCH of lactose standard solution

RESULTS AND DISCUSSION

The LCMS method was developed and verfied at system suitability and LOQ levels.

To assess the linearity, seven standard curves for lactose were constructed, plotting concentration (ng/ml) versus area and showed good linearity on the 3.089-51.490 ng/ml range. The representative linear equation was y = 2.02e+003 x + 1.4e+003 (r = 0.9945), where x is concentration and y is the peak area. The correlation coefficient r was 0.9945, indicating good linearity (Figure 4).

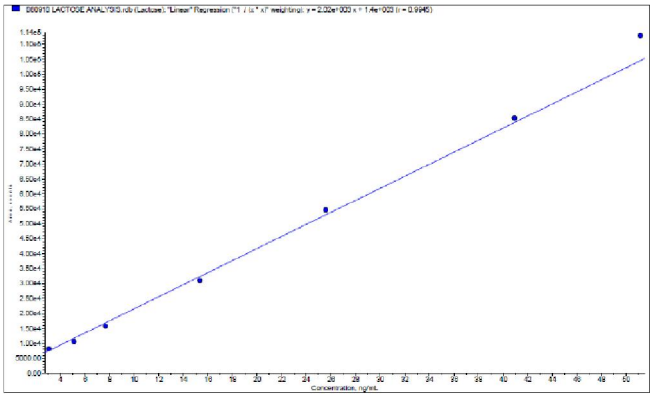

Figure 4 : Calibration curve of lactose standard solution.

CONCLUSION

The study tested the LCMS method development of swab and air sampling to determine lactose levels in samples collected in production premises. The LCMS method was optimized, and the test of lower limit of quanitation indicated that the method met the established acceptance criteria. The concentration of swab and air samples was found to be below detection limit. The analysis method used in cleaning validation must be sensitive and selective in order to detect and identify trace contaminants, and quantitative to report the extent of the contamination. LCMS meets all of these criteria and it is fast, much faster than HPLC, the method most commonly used in cleaning validation.

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