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THOMSON REUTERS
[IDSTM: January 2017]
IC™ Value: 3.00
ISSN: 2277-9655
Impact Factor: 4.116
CODEN: IJESS7

IJESRT

INTERNATIONAL JOURNAL OF ENGINEERING SCIENCES & RESEARCH TECHNOLOGY

STANDARDIZATION OF HPTLC METHOD FOR THE DETECTION OF FORMALDEHYDE IN MILK

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ABSTRACT

The present piece of work deals with the development of a simple, rapid, accurate reverse phase high performance thin layer chromatographic method for the detection of formaldehyde in milk. The Standardization of the method was based on simulation parameters such as mobile phase, stationary phase and saturation time of the solvent chamber. The optimized stationary phase was aluminium baked pre-coated silica gel 60 F254s HPTLC plates, mobile phase: chloroform: dichloromethane: diethyl ether (4:5:0.8, v/v/v), saturation time: 5 min. The method was validated by testing its linearity, precision, accuracy, limit of detection and quantification. In our country milk is adulterated with several adulterants. The preservatives added in milk such as formaldehyde is although beneficial but can have several side effects also. Milk samples collected from different region of Agra city were tested for the presence of formaldehyde in milk. Maximum adulteration was found to be in the milk samples collected from Rambagh region.

KEYWORDS: Adulteration, Formaldehyde, HPTLC, Validation.

INTRODUCTION

Milk is universally recognized complete diet due to its essential components. It is recommended as compulsory part of daily diet for the expectant mothers as well as growing children (Shah et al, 1982). Today, the quality of food is hardly maintained at consumer level which plays a very important role in maintaining healthy society. Adulteration of food items using several chemical substances or impurities that can cause severe damage to the living system is a mind-boggling problem in society. Adulterants have an impact over the respiratory system, digestive system, nervous system, brain and also heart.

In our country, milk is adulterated with several adulterants (Izhar et al, 1991; Summer et al, 2003; Karoui et al, 2007). There are several dairies; companies that made packed milk to be sold in market but these all are added with preservatives in order to keep them fresh and capable of being used even after 4-5 days. These preservatives are although beneficial but can have several side effects also. Extension of milk with a low value ingredient (watering of milk, milk of different species, addition of whey, etc) also known as “economic adulteration” has been often practiced (Ullah et al, 2005; Oancea et al, 2009). Adulteration of milk with urea has been very common in our country. The adulteration of milk by formalin and carbonate & bicarbonate is another factor by which the milk quality is debased (Shah et al, 1982; Ayub et al, 2007).

In the present study, a rapid, reliable and cost effective high performance thin layer chromatographic method has been standardized for the estimation of formaldehyde in milk samples. Aluminium coated silica gel 60 F_{254} HPTLC plates were used for the analysis of formaldehyde content in different milk samples. The standardization of the method was based on simulation parameters of mobile phase, stationary phase and saturation time. The proposed method is simple rapid specific and was successfully employed for quality and quantity monitoring of formaldehyde content in edible products.

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MATERIALS AND METHODS

Chemicals and reagents:

Formaldehyde, Dimedone, Chloroform and Dichloro methane. All the chemicals and solvents used were of HPLC grade and purchased from Sigma Aldrich Chemicals Pvt. Ltd, New Delhi.

Standard and Sample Preparation:

Standard solution of formaldehyde was freshly prepared by dissolving formaldehyde (2.0 mg/ml) in methanol followed by sonication for 10 min. Dimedone solution (2.0 mg/ml) was prepared in methanol and sonicated for 10 min.

HPTLC analysis:

HPTLC analysis was performed on a computerized densitometer scanner 3 connected to a PC running WinCATS planar chromatography manager version 1.4.4 (Camag, Switzerland); an auto sampler Linomat V using 100 µL syringe, connected to a nitrogen cylinder; and a UV scanner. HPTLC (Silica gel F_{254}) plate contains different tracks of sample and standards (2-16µl) were developed in twin trough chamber using optimized solvent system under following conditions: band width 8 mm; distance between bands 3 mm; gas flow rate 10s/µL. UV scanner was set for the maximum light optimization with the following settings: slit dimension, 6.00 mm × 0.30 mm, scanning speed, 20 mm/s; data resolution, 100 µm/step. Remaining parameters were left as default settings. Regression analysis and statistical data were automatically generated by the WinCATS software.

Test samples (5µl) were spotted along with standard solution of formaldehyde + Dimedone (2-16µl) respectively on pre-coated silica gel 60 F_{254} plate (20 cm x 10 cm). The plate was developed in optimized mobile phase and scanned at 254 nm for the estimation of formaldehyde in milk samples. Peak area was noted and concentration was determined by comparing the area of standard solution from calibration curve.

RESULTS AND DISCUSSION

Preliminary tests on silica gel, alumina and cellulose coated HPTLC plates indicated that silica gel layer gave the best resolution of the peak for formaldehyde. Therefore, all subsequent analyses were done on silica gel layers. Optimization of solvent system has been achieved based on simulation in R_f values based on different polarity as well as the saturation time.

The chromatographic profile and peak of formaldehyde + dimedone was identified using optimum solvent system Chloroform: Dichloro methane: Diethyl ether (4:5:0.8, v/v/v) at R_f value of 0.73 ± 0.01 and there was no overlap with any other analyte of the sample at 254 nm (Fig 1). Presence of formaldehyde in milk samples were also confirmed by recording the UV spectra of standard formaldehyde and analyte present in samples (Fig 2).

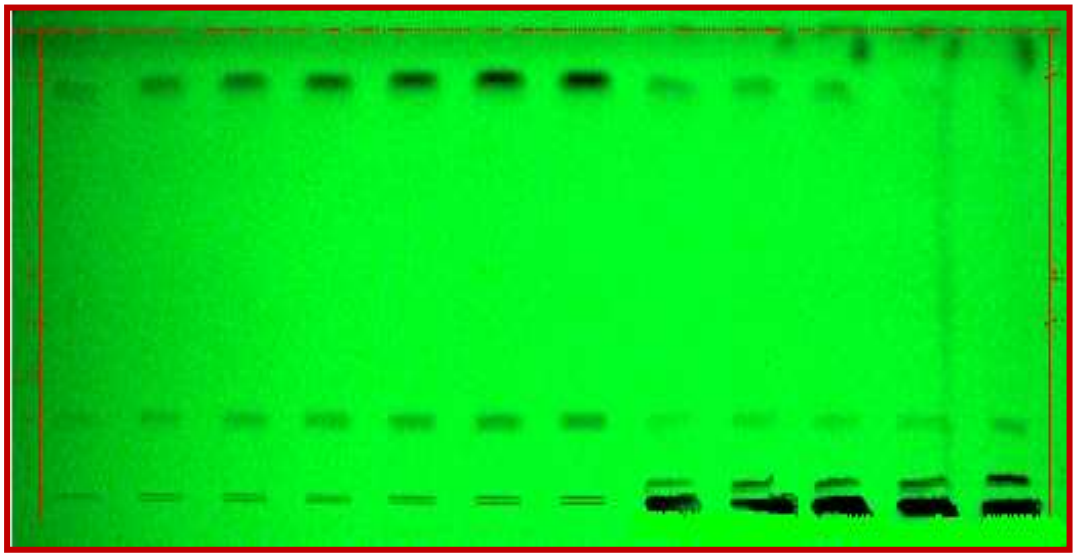

Fig 1. HPTLC Plate image recorded at 254 nm for formaldehyde in milk samples

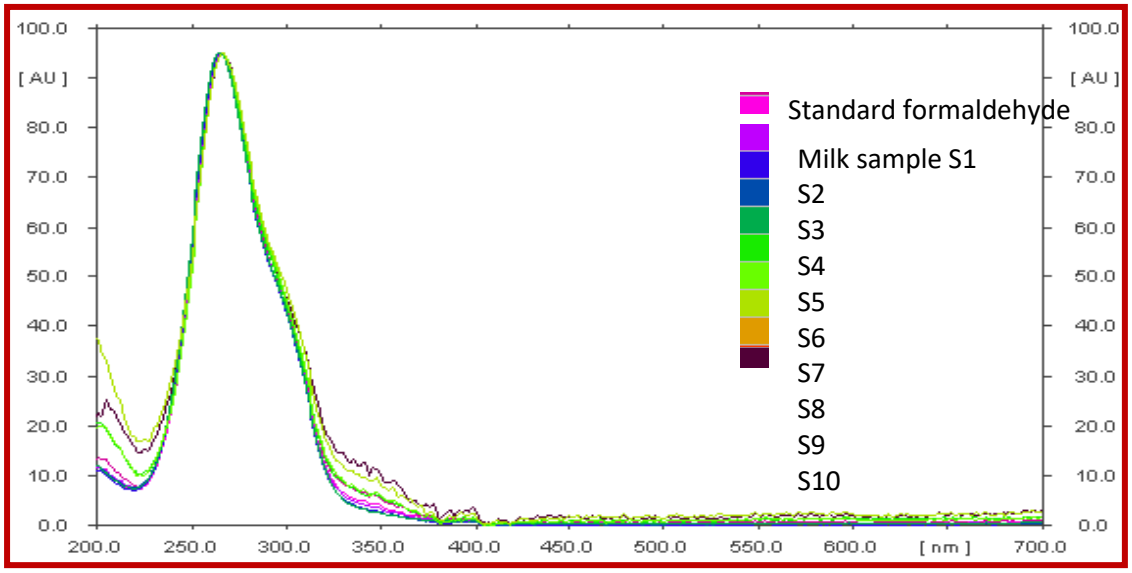

Figure 2 UV Spectra of standard Formaldehyde and test samples indicating adulteration with Formaldehyde

Validation of the method

The developed chromatographic method was validated according to ICH guidelines. Validation parameters include linearity, accuracy in terms of recovery %, limit of detection and quantification and precision. Selection of wavelength (254 nm) is specific for the detection of formaldehyde and enabled its detection at R_f value of 0.73 ± 0.01.

Linearity

Linearity of the target adulterant was obtained in the range 4-32µg of formaldehyde-dimedone adduct. Linearity curve was obtained with winCATS software (figure 5.34) with the following parameters slope: 1.455, intercept: 1549.133, regression coefficient (r^2) = 0.99692, standard deviation = 2.84%. 20µl of test samples were also loaded on the plate and quantified. The amount of formaldehyde in samples was computed from the calibration curves with the help of winCATS software (Fig 3).

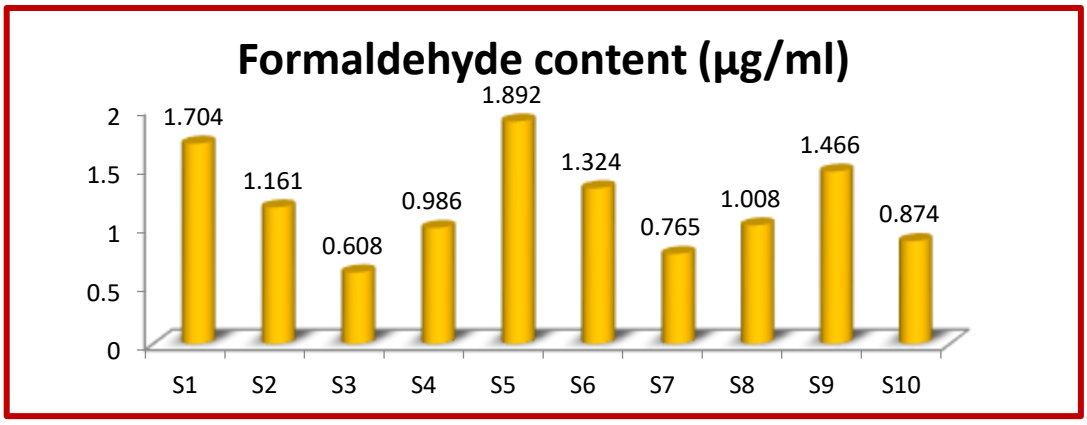

Figure 3 Formaldehyde content as found in different milk samples

Accuracy

Accuracy in terms of % recovery was measured for this method. This method is accurate up to 95.20 – 97.14 %.

LOD and LOQ

The limit of detection (LOD) and quantification (LOQ) was found to be 6.44 and 19.51 pg, respectively.

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Precision

Precision (repeatability) was determined by running a minimum of six analyses and the coefficient of variability was found to be 1.812%.

CONCLUSION

An accurate reverse phase HPTLC method has been developed for the simultaneous determination of formaldehyde in milk samples. The proposed HPTLC method was found to be simple, sensitive, rapid, specific, accurate, precise, reproducible and repeatable for qualitative and quantitative estimation of formaldehyde in milk samples. Standard conditions have been optimized, based on simulation in R_f values under different experimental conditions (stationary phase, polarity of mobile phase and saturation time). The proposed method was validated in terms of Linearity, precision, accuracy, limit of detection and limit of quantification.

ACKNOWLEDGEMENT

The authors are grateful to Prof. P. K. Kalra, Director, Prof. Sahab Das, Head, Department of Chemistry, Dayalbagh Educational Institute, Dayalbagh, Agra. The authors are also thankful to Ministry of Human Resource and Development, Govt. of India, for rendering financial assistance.

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