Artificial Neural Network Modeling & Standardization of HPTLC Method for the Estimation of Cholesterol in Edible Oils

J. K. Arora[1]* and M. M. Srivastava[2]

Abstract - **ANN model was designed to predict optimum R_f values obtained from high performance thin layer chromatography of cholesterol in market available edible oils. A single-layer Artificial Neural Network model was obtained based on R_f values under experimental conditions of polarity of mobile phase, stationary phase and saturation time by combining back propagation (BP) with principle component analysis. A hyperbolic tangent was used as transfer function for input and output layer. The Levenberg–Marquardt algorithm (LMA) was applied, giving a minimum mean squared error (MSE) for training and cross validation as 1.16955E-29 and 0.007784122 respectively. ANN predictions of Rf values for different oils were found in harmony with experimental findings. The peanut oil contains highest (0.71%) while coconut oil contains lowest (0.15%) cholesterol level.**

Keywords: Artificial Neural Network, Cholesterol, Edible oils, HPTLC

I. INTRODUCTION

The development of instrumental methods for the identification and quantification of individual components in food and beverages has become extremely important for establishing the oil quality and their genuineness. Commonly used techniques for the analysis of constituents of edible oils are GC and HPLC [1]. High Performance Thin Layer Chromatography (HPTLC) is recently introduced technique for the analysis of food products without chemical treatment of the sample and has the advantages of simplicity, speed, reproducibility and cost effectiveness [2]. It is an offline technique: the subsequent steps are relatively independent, allowing parallel treatment of multiple samples during chromatography, derivatization and detection. To achieve an optimum management for the development of the method, the concept of modeling for an efficient operation is essentially required. A high quality representative model can provide a favorable solution to the process control. It is likely to explain the real process performance developing a continuous control strategy for such type of methods. Artificial Neural Network (ANN) has been successfully employed in various environmental [3-6], analytical & engineering processes [7].

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The present work reports standardization of HPTLC method for the estimation of cholesterol in six popular edible oils (Coconut, Soybean, Peanut, Mustard, Sunflower and Taramira). Prediction of R_f values in different experimental conditions has been carried out using single- layer ANN model. Pursuing benchmark comparisons of BP algorithms, a study was conducted to determine the optimal network structure. The output obtained from the ANN modeling was compared with the experimental data.

II. MATERIALS AND METHODS

A. CHEMICALS AND REAGENTS

Pure cholesterol was obtained from E. Merck (Darmstadt, Germany). Six popular edible (Coconut, Soybean, Peanut, Mustard), less popular (Taramira) and cholesterol free (Sunflower) oils available in the market were considered for the study. HPTLC plates (silica gel 60 F254, alumina 60 F254, 20×10 cm) purchased from E. Merck (Darmstadt, Germany) were used for analysis. Plates were developed in a chromatographic chamber using optimized solvent system comprising of n-Hexane- Diethyl ether- MeOH. The solvent was allowed to migrate up to a height of 80 mm from the lower edge of the plate and then dried it.

B. SAMPLE PREPARATION AND ANALYSIS

Standard solution of cholesterol was freshly prepared by dissolving cholesterol (0.05 mg/ml) in toluene. Edible oils were mixed with toluene and sonicated for 30 minutes for proper mixing and then injected on the HPTLC plates for the analysis. HPTLC system (Camag, Muttanz, Switzerland) consisted of a TLC scanner which is connected to a PC running WinCATS; an auto sampler Linomat V using 100µL and 500µL syringes, connected to a nitrogen cylinder; a UV scanner. Each HPTLC plate contains different tracks of samples and standards under following conditions: band width 6mm; distance between bands 3mm; application volume of standard cholesterol 2–14µL; gas flow rate10s/µL. UV scanner was set for the maximum light optimization with the following settings: slit dimension, 4.00mm × 0.30mm, micro; scanning speed, 20mm/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.

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III. RESULTS AND DISCUSSIONS

A. QUANTITATIVE ANALYSIS OF CHOLESTEROL AND THEIR VALIDATION

The chromatographic profile of cholesterol in the samples was simple, showing cholesterol as the main component. Presence of cholesterol was confirmed by recording the spectra of standard cholesterol and edible oil samples in the range of 200-400 nm. Peak of cholesterol was identified in n-Hexane-Diethyl ether- MeOH (5:2:1.5, v/v/v) with the R_f value of 0.66 ± 0.01 and there was no overlap with any other analyte of the sample at 200 nm.

The developed chromatographic method was validated. Validation parameters include selectivity, linearity, accuracy in terms of recovery %, limit of detection and quantification and precision. Selection of wavelength (200 nm) is specific for the detection of cholesterol and enabled its detection at R_f value of 0.66 ± 0.01. The linearity of the proposed method was confirmed in the range of 100-700 ng of standard cholesterol. This range was suitable for the determination of cholesterol content in edible oils. A linear regression of the data points for standard cholesterol is resulted in a calibration curve with the equation $Y = 644.436 + 652.7908x$ [regression coefficient (r^2) = 0.99831, standard deviation (S.D.) = 2.13%] (Fig.1). The correlation coefficient was found to be greater than 0.998 which manifests a linear relationship between concentration and the peak area.

Cholesterol content in edible oils was found to be in the range of 150-710 ng (Table 1). The peanut oil contains highest (0.71%) while coconut oil contains lowest (0.15%) cholesterol level. The calibration curve was accurate within the specified concentration range with a mean recovery of 90.32 ± 1.04%. The limit of detection (LOD) and quantification (LOQ) was found to be 10 and 32 ng respectively. Precision (repeatability) was determined by running a minimum of four analyses and the coefficient of variability was found to be 1.662 %.

B. OPTIMIZATION OF THE ANN STRUCTURE

Optimization of the HPTLC method for the estimation of cholesterol in edible oils was calculated in through the real HPTLC experiments as a function of solvent system, stationary phase and saturation time. During training, the output vector is computed by a forward pass in which the input is propagated forward through the network to compute the output value of each unit. The output vector is then compared with the desired vector which resulted into error signal for each output unit. In order to minimize the error, appropriate adjustments were made for each of the weights of the network. After several such iterations, the network was trained to give the desired output for a given input vector. The single layer network structure included fifty hidden neurons with 5000 Epoch, describing the R_f value of cholesterol (Fig. 2). The tanh axon was considered transfer function with 0.7 momentums. The performance of network simulation was evaluated in terms of mean square error (MSE) criterion. The MSE for the training and cross validation data sets as 1.16955E-29 and 0.007784122 respectively. The developed network model was examined for its ability to predict the response of experimental data not forming the part of the training program. Fig.3 shows the result obtained by the neural network simulation for both the training and cross validation data sets. ANN predictions of Rf values for different oils were found in harmony with experimental findings (Fig.4).

IV. CONCLUSION

The Levenberg–Marquardt algorithm (LMA) was found best of BP algorithms with a minimum mean squared error (MSE) for training and cross validation as 1.16955E-29 and 0.007784122 respectively. The proposed ANN model is proved meaningful supplement for the conventional and complicated experiments in the prediction of R_f values and highlights the possibility of the prediction of the optimum conditions for the quantification of any particular gradient in the edibles, where lab experiments have not been conducted.

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Table 1: Cholesterol levels in edible oils

Edible oils	**Cholesterol content [%]**
Coconut	0.15
Mustard	0.28
Taramira	0.32
Soyabean	0.42
Sunflower	0.56
Peanut	0.71

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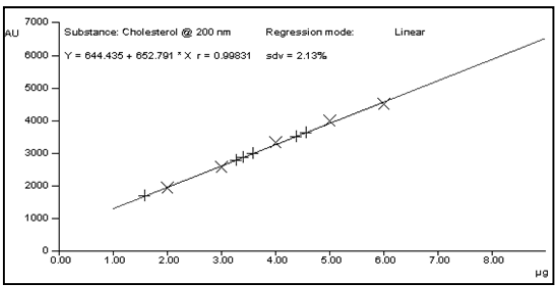


Figure 1: Calibration curve of cholesterol

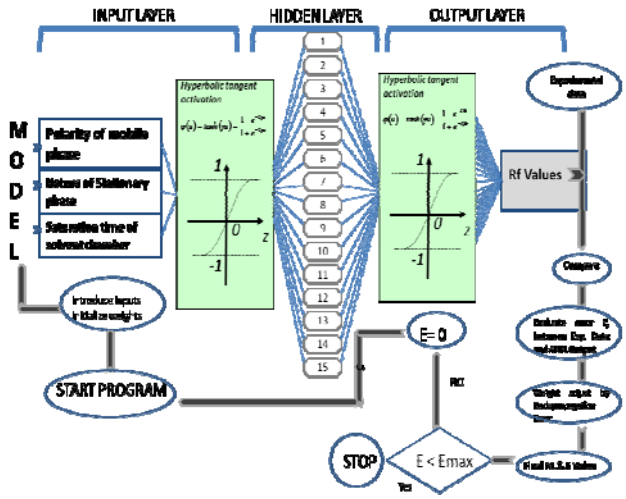
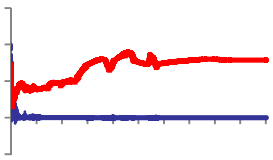


Figure 2: Single layer Neural Network structure describing the R_f value of cholesterol.



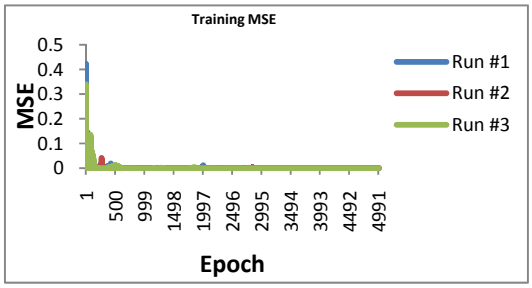
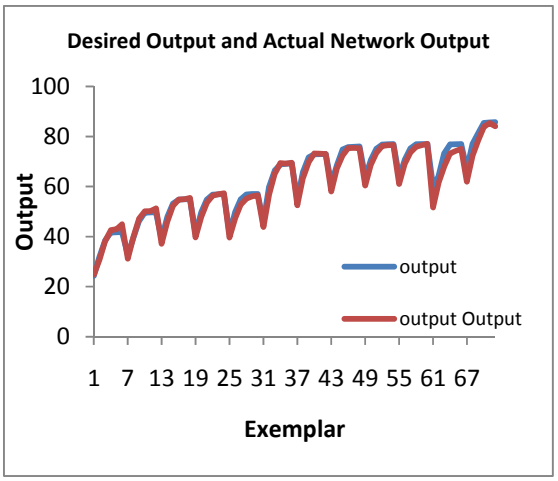
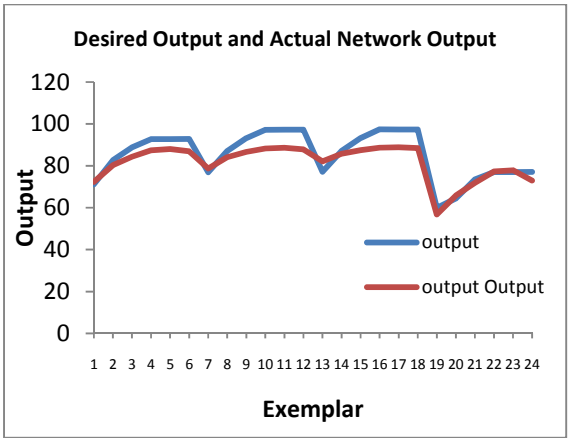


Figure 3: (a, b): Graphical representation of MSE value with 5000 Epoch- Average MSE & Training MSE.



a)



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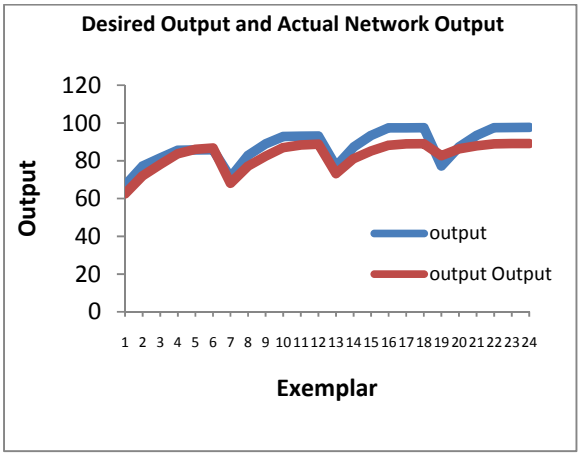

c)

Figure 4: Comparison of Desired output and Network output for: a) Training; b) Cross validation; c) Testing [Y axis, 1/100 unit = 1 unit].

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