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Spectrometric Multi Component Determination of Maltol, Ethyl Maltol, Vanillin and Ethyl Vanillin in Foods by Multi Linear Regression Calibration, Classical Least Square and Inverse Least Square Methods

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Multi linear regression calibration (MLRC), classical least square (CLS) and inverse least square (ILS) were proposed for simultaneous determination quantitative analysis of quartet mixture consist of maltol (MAL), ethyl maltol (EMA), vanillin (VAN) and ethyl vanillin (EVA) in commercial preparations. In the chemometric techniques, the concentration matrix was prepared by using the synthetic mixtures containing these food additives. The absorbance matrix corresponding to the concentration matrix was obtained by measuring the absorbances at 9 wavelengths in the range 260-340 nm for the zero-order spectra. Chemometric calibrations were constructed by using absorbance matrix and concentration matrix for the prediction of the unknown concentrations of maltol, ethyl maltol, vanillin and ethyl vanillin in their mixture. The numerical values were calculated by using "MAPLE 7" and "EXCEL" software. The obtained results were statistically compared with each other.

Key Words: Determination, Multi linear regression calibration, Classical least square, Inverse least square, Maltol, Ethyl maltol, Vanillin, Ethyl vanillin,

INTRODUCTION

Maltol (MAL), ethyl maltol (EMA), vanillin (VAN) and ethyl vanillin (EVA) are important materials used in the food industry. These compounds are widely used in dairy and artificial dairy products such as candies, cookies, chocolate and beverages[1]. These compounds can enhance the scent of foods, they are synthetic perfumes and food additives. If large amounts of these flavour enhancers are ingested they cause headaches, nausea *etc*. Because of the volatility, instability and insoluble properties in water, they are not easily directly determined in solution[2]. Consequently, it is important to determine their contents in foods.

The analytical methods for determination of maltol, ethyl maltol, vanillin and ethyl vanillin, include high performance liquid chromatography methods[3], electrochemical methods[4,5], UV-vis spectrometry methods[6,7] and gas chromatography-mass spectrometry methods[7-10], have been reported.

In recent years, chemometric calibration techniques can be summarized as multiple linear regression (MLR) classical least square (CLS) and inverse least squares (ILS) calibrations, principal component regression (PCR) and partial least regression (PLS) techniques[11-15]. Chemometric calibration techniques in spectral analysis is gaining importance in the quality control of food additives in mixtures and commercial products formulations of two or more food additives with overlapping spectra. Several researchers used these techniques for the simultaneous analysis binary and ternary mixture[16-18]. All the multivariate approaches are useful for the resolution of spectral band overlapping in quantitative determination. In the multivariate analysis, a calibration is build from spectral response values for a set of standard samples as known concentrations corresponding to the analytes of interest. The obtained calibration is used to predict the component concentrations from the sample spectrum. The multivariate calibration-prediction techniques use the full spectrum, full automation, multivariate data analysis and the reduction of noise and the advantages of the selection of the calibration model. In addition these multivariate calibrations do not need any separation procedure, they are cheap, easy to apply and sensitive. For these reasons these multivariate techniques are popular today.

In this study, the MLR, CLS and ILS calibration models were described for the spectrometric resolutions of the samples, containing maltol, ethyl maltol, vanillin and ethyl vanillin. A computer program MAPLE 7 was used to perform the construction of multivariate calibrations-predictions. These calibrations were tested for the synthetic mixtures containing the four food additives and they were applied to the simultaneous resolutions of maltol, ethyl maltol, vanillin and ethyl vanillin in a commercial product, marketed in Isparta, Turkey.

EXPERIMENTAL

Absorbance measurements were carried out by using a Perkin-Elmer Lambda 20 double beam UV-visible spectrophotometer interfaced to an IBM SX-486 microcomputer for the spectral acquisition provided with a UV-Win Lab software and subsequent manipulation of the experimental data. The absorbance measurements were carried out in two matching quartz 1.0 cm cells with a 1 mm path length. The absorption spectra were recorded between 200 and 350 nm with an interval of 0.1 nm between each two points and were contrasted with the corresponding blanks. All these data were recorded and used for calculations.

All solvents and reagents were of analytical reagent grade unless otherwise indicated. Maltol, ethyl maltol, vanillin and ethyl vanillin were supplied by Fluka (Fluka Chemie GmbH CH-9471 Germany). Stock solutions of maltol, ethyl maltol, vanillin and ethyl vanillin 5 mg 100 mL^{-1} of each, were prepared by dissolving each of the crystalline compounds in 95 % ethanol. Working solutions of 250 mgL^{-1} were prepared by dilution with double distilled water before use. Britton-Robinson buffer solutions was prepared in such a way that 2.3 mL glacial acetic acid, 2.7 mL phosphoric acid and 5.0 g boric acid dissolved in water then by dilution with water to 1.0 L. 50 mL portion of this solution was taken and the pH was adjusted between 2 and 6 by addition of the appropriate amount of 2.0 M KOH solution.

Procedure: Suitable amount of working solutions of maltol, ethyl maltol, vanillin and ethyl vanillin or their mixtures, were transferred to 25 mL volumetric flask followed by the addition of 5.0 mL Britton-Robinson buffer solution (pH 2.87), diluted to the mark with the doubly distilled water and mixed well.

Several commercial food samples were purchased from the local market in Isparta city. The food samples were ground to a fine powder with a mortar and pestle. 20.0 g of this powder and 50 mL of anhydrous ethanol were placed into a 100 mL Erlenmeyer flask and shaken by a laboratory shaker for 2 h. This mixture was then transferred to a 10 mL centrifuge tube and centrifuged 5000 rpm for 5 min. The clear part of solution in the tube was used for analysis. Suitable amount of this sample was transferred into a 25 mL flask, added 5.0 mL 95 % ethanol and then the solution was analyzed by the analytical procedure as described above.

Multi linear regression calibration method: Multi linear regression calibration algorithm[19] for the quantitative analysis of ternary or multi mixtures is based on the application of linear algebra to linear regression function at a multi point set of selected wavelengths in the working spectral range. Multi linear regression calibration algorithm is explained in the following steps.

If the absorbance values of a mixture of four analytes (X, Y, Z and Q) are measured at n wavelengths ($\lambda_i = 1, 2, \ldots, n$), the following set of function can be written for a four-component analysis:

$$\begin{array}{l} A_{mix_1} = m_{X_1} \cdot C_X + m_{Y_1} \cdot C_Y + m_{Z_1} \cdot C_Z + m_{Q_1} \cdot C_Q + e_{XYZQ_1} \\ A_{mix_2} = m_{X_2} \cdot C_X + m_{Y_2} \cdot C_Y + m_{Z_2} \cdot C_Z + m_{Q_2} \cdot C_Q + e_{XYZQ_2} \\ \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \\ \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \\ A_{mix_n} = m_{X_n} \cdot C_X + m_{Y_n} \cdot C_Y + m_{Z_n} \cdot C_Z + m_{Q_n} \cdot C_Q + e_{XYZQ_n} \end{array} \quad (1)$$

where A_{mix_1}, A_{mix_2}, … and A_{mix_n} are the absorbances of the mixture of X, Y, Z and Q analytes at selected wavelengths (from λ_1 to λ_n); m_{X_1}, m_{X_2}, …, $m_{X_{1n}}$, m_{Y_1}, m_{Y_2}, …, m_{Y_n}, m_{Z_1}, m_{Z_2}, …, m_{Z_n} and m_{Q_1}, m_{Q_2}, …, m_{Q_n} are the slopes of n linear regression functions of X, Y, Z and Q, corresponding to selected wavelengths, respectively; and e_{XYZQ_1}, e_{XYZQ_2}, …, e_{XYZQ_n} are the sum of intercepts of linear regression functions at n wavelengths ($e_{XYZQ_n} = e_{X_n} + e_{Y_n} + e_{Z_n} + e_{Q_n}$).

In the matrix terms, the above multi-equation system (1) can be formulated as:

$$\begin{bmatrix} A_{mix_1} \\ A_{mix_2} \\ \ldots \\ \ldots \\ A_{mix_n} \end{bmatrix} = \begin{bmatrix} m_{X_1} & m_{Y_1} & m_{Z_1} & m_{Q_1} \\ m_{X_2} & m_{Y_2} & m_{Z_2} & m_{Q_2} \\ \ldots & \ldots & \ldots & \ldots \\ \ldots & \ldots & \ldots & \ldots \\ m_{X_n} & m_{Y_n} & m_{Z_n} & m_{Q_n} \end{bmatrix} \cdot \begin{bmatrix} C_X \\ C_Y \\ C_Z \\ C_Q \end{bmatrix} + \begin{bmatrix} e_{XYZQ_1} \\ e_{XYZQ_2} \\ \ldots \\ \ldots \\ e_{XYZQ_n} \end{bmatrix} \quad (2)$$

which can be simplified to

$$\begin{bmatrix} A_{mix_1} - e_{XYZQ_1} \\ A_{mix_2} - e_{XYZQ_2} \\ \cdots \\ \cdots \\ A_{mix_n} - e_{XYZQ_n} \end{bmatrix} = \begin{bmatrix} m_{X_1} & m_{Y_1} & m_{Z_1} & m_{Q_1} \\ m_{X_2} & m_{Y_2} & m_{Z_2} & m_{Q_2} \\ \cdots & \cdots & \cdots & \cdots \\ \cdots & \cdots & \cdots & \cdots \\ m_{X_n} & m_{Y_n} & m_{Z_n} & m_{Q_n} \end{bmatrix} \cdot \begin{bmatrix} C_X \\ C_Y \\ C_Z \\ C_Q \end{bmatrix} \tag{3}$$

in a compact form

$$(A_{mix} - e_{XYZQ})_{nx_1} = K_{nx_4} \cdot C_{4x_1} \tag{4}$$

The matrix of the slope values is called the matrix K:

$$K_{nx_4} = \begin{bmatrix} m_{X_1} & m_{Y_1} & m_{Z_1} & m_{Q_1} \\ m_{X_2} & m_{Y_2} & m_{Z_2} & m_{Q_2} \\ \cdots & \cdots & \cdots & \cdots \\ \cdots & \cdots & \cdots & \cdots \\ m_{X_n} & m_{Y_n} & m_{Z_n} & m_{Q_n} \end{bmatrix} \tag{5}$$

The matrices, $(A_{mix} - e)_{nX_1}$ and K_{nX_4}, are multiplied by the transpose $(K')_{4X_n}$ of the matrix K_{nX_4} and it can be written as:

$$(K')_{4X_n}(A_{mix} - e)_{nX_1} = (K')_{4X_n} K_{nX_4} \cdot C_{4X_1} \tag{6}$$

The concentration of the X, Y, Z and Q compounds in ternary mixture can be calculated by using the following formula:

$$C_{4X_1} = [(K')_{4X_n} K_{nX_4}]^{-1}_{4X_4} \cdot [(K')_{4X_n} (A_{mix} - e_{XYZQ})_{nX_1}] \tag{7}$$

In this case, the MLRC model contains the use of linear algebra, also known as matrix mathematics. This calibration model can be applied to the multi resolution of multi-component mixture system containing n compounds.

Classical least squares method: In this approach, the method is based on the use the absorptivity values at the selected wavelengths for the spectrometric quantitative analysis multi-component mixture system containing n compounds[13]. Absorptivity, A_1^1 (1 %, 1 cm), values of four compounds X, Y, Z and Q are calculated by using the absorbance measured at the selected wavelengths in zero-order spectra for each of the compounds in quartet mixture. By using values, systems of equations with n unknowns were written for the compounds in the quartet mixture. Matrix notation greatly simplifies matters and easily solves the system of equations with four unknowns, as shown below:

$$\begin{bmatrix} A_1 \\ A_2 \\ \cdots \\ \cdots \\ A_n \end{bmatrix} = \begin{bmatrix} \alpha_1 & \beta_1 & \gamma_1 & \phi_1 \\ \alpha_1 & \beta_1 & \gamma_1 & \phi_1 \\ \cdots & \cdots & \cdots & \cdots \\ \cdots & \cdots & \cdots & \cdots \\ \alpha_n & \beta_n & \gamma_n & \phi_n \end{bmatrix} \times \begin{bmatrix} C_X \\ C_Y \\ C_Z \\ C_Q \end{bmatrix} \tag{8}$$

where $A_1, A_2, \ldots, A_n$ represent the absorbance of solution of mixtures of X, Y, Z and Q, $\alpha_{1, 2, \ldots n}$, $\beta_{1, 2, \ldots n}$, $\gamma_{1, 2, \ldots, n}$ and $\phi_{1, 2, \ldots, n}$ denote the values calculated for X, Y, Z and

Q, respectively at λ_1, λ_2, …. and λ_n. C_X, C_Y, C_Z and C_Q are the concentration of X, Y, Z and Q, respectively in g/100 mL. Using the similar procedure described in MLRC method, this matrix was solved and it was determined, the concentration of X, Y, Z and Q in their mixture.

Inverse least squares method: Inverse least-squares (ILS)[13], sometimes known as P-matrix calibration, is so called because, originally, it involved the application of multiple linear regression (MLR) to the inverse expression of the Beer-Lambert Law of spectroscopy:

$$C = P \times A \tag{9}$$

The above equation can be written as a linear equation system as follows:

$$\begin{aligned} C_1 &= P_{11}A_1 + P_{12}A_2 + \ldots + P_{1W}A_W \\ C_2 &= P_{21}A_1 + P_{22}A_2 + \ldots + P_{2W}A_W \\ C_3 &= P_{31}A_1 + P_{32}A_2 + \ldots + P_{3W}A_W \\ \ldots &\quad \ldots \quad \ldots \quad \ldots \quad \ldots \\ C_C &= P_{C_1}A_1 + PW_2A_2 + \ldots + P_{CW}A_W \end{aligned}$$

where A_W is the absorbance wth wavelength, P_{CW} is the calibration coefficient for the cth component at the wth wavelength, C_C is the concentration of the cth component.

RESULTS AND DISCUSSION

Fig. 1 shows the absorption spectrum for individual components as well as their corresponding quartet mixtures ranging from 200-350 nm. As shown, there is a high spectra overlap, which makes it difficult for simultaneous determination of the analytes in a mixture, without any sample or data manipulation. In order to solve this problem, the MLRC method was applied to the multi resolution of the four component mixture system of the subject matter compounds. As an alternative, the CLS and ILS methods were used to solve the problem. For this purpose, the standard series of solutions of MAL, EMA, VAN and EVA (1-8 μg/mL) in 0.1 M HCl were prepared. Their absorption spectra were recorded over the wavelength range 200-350 nm against blank (0.1 M HCl). In order to validate the method, the synthetic mixture solutions of MAL, EMA, VAN and EVA were prepared according to working range of the individual compounds.

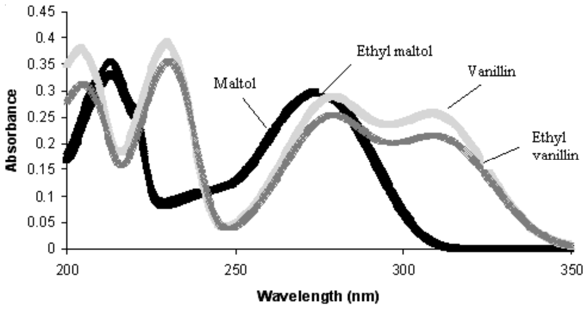

Fig. 1. Absorbance-wavelength curves for maltol, ethyl maltol, vanillin and ethyl vanillin and their mixture in 0.1 HCl

MLRC method: The nine-wavelength set at the critical points, which correspond to the maximum, shoulder and minimum in the spectral range 200-350 nm were selected for the construction of the individual linear regression for MAL, EMA, VAN and EVA in the quartet mixture. Nine linear regression equations of MAL, EMA, VAN and EVA for each compound were obtained by measuring the zero-order absorbance values at the wavelengths set. Results were presented in Table-1.

TABLE-1
RESULTS OF MLRC METHOD

λ_i	Equations
260	$A_{mix_1} = 0.0442C_{MAL} + 0.0366C_{EMA} + 0.0245C_{VAN} + 0.0228C_{EVA} + 0.0133$
270	$A_{mix_2} = 0.0588C_{MAL} + 0.0507C_{EMA} + 0.0452C_{VAN} + 0.0422C_{EVA} + 0.0333$
280	$A_{mix_3} = 0.0561C_{MAL} + 0.0503C_{EMA} + 0.0540C_{VAN} + 0.0494C_{EVA} + 0.0383$
290	$A_{mix_4} = 0.0364C_{MAL} + 0.0340C_{EMA} + 0.0462C_{VAN} + 0.0507C_{EVA} + 0.0288$
300	$A_{mix_5} = 0.0134C_{MAL} + 0.0127C_{EMA} + 0.0451C_{VAN} + 0.0430C_{EVA} + 0.0208$
310	$A_{mix_6} = 0.0000C_{MAL} + 0.0018C_{EMA} + 0.0480C_{VAN} + 0.0406C_{EVA} + 0.0183$
320	$A_{mix_7} = 0.0000C_{MAL} + 0.0000C_{EMA} + 0.0381C_{VAN} + 0.0339C_{EVA} + 0.0140$
330	$A_{mix_8} = 0.0000C_{MAL} + 0.0000C_{EMA} + 0.0196C_{VAN} + 0.0185C_{EVA} + 0.0066$
340	$A_{mix_9} = 0.0000C_{MAL} + 0.0000C_{EMA} + 0.0061C_{VAN} + 0.0063C_{EVA} + 0.0003$

CLS method: Absorptivity, A_1^1 (1 %, 1 cm), values of all the four compounds, MAL, EMA, VAN and EVA were calculated by using the absorbance's measured at the above mentioned nine wavelengths in the zero-order spectra for each of the compounds in quartet mixture. By using values, a system of equations with nine unknowns was written for the compounds in the quartet mixtures as described above. Using the procedure explained, this matrix was solved and it was determined the concentration of MAL, EMA, VAN and EVA in their mixture.

ILS method: As explained in the CLS technique, the regression coefficient matrix (P) mentioned in the multivariate calibration-prediction techniques for ILS technique was computed by means of the training set and its absorbance data for nine points in the wavelength range 200-350 nm. When the calculated P matrix was replaced in the expression below, the following was obtained:

$$\begin{bmatrix} C_{MAL} \\ C_{EMA} \\ C_{VAN} \\ C_{EVA} \end{bmatrix} = \begin{bmatrix} 0.3235, 0.3507, 0.4072, 0.4771, 0.5538, 0.6084, 0.5847, 0.6052, 0.4915 \\ 0.3639, 0.3636, 0.3334, 0.2984, 0.2508, 0.2022, 0.0801, 0.3553, 0.0915 \\ 0.4059, 0.4297, 0.4977, 0.6404, 0.8298, 1.0350, 1.2400, 1.3710, 1.3630 \end{bmatrix} \begin{bmatrix} A_1 \\ A_2 \\ A_3 \\ \cdots \\ \cdots \\ A_9 \end{bmatrix}$$

In this expression, C_{MAL}, C_{EMA}, C_{VAN} and C_{EVA} are the concentrations MAL, EMA, VAN and EVA, respectively. The prediction of unknown concentrations of MAL, EMA, VAN and EVA in samples realized with the resolution of the above system.

To check the validity of the calibration models, the multi resolution of the synthetic mixtures containing various concentrations of MAL, EMA, VAN and EVA was carried out by the MLRC, CLS and ILS methods. Results were summarized in Table-2. The means recoveries and their relative standard deviation of the methods were computed.

The predictive ability of a model can be defined by various ways. The most general expression is the standard error of prediction (SEP) which is given below equation:

$$SEP = \sqrt{\frac{\sum_{i=1}^{N}(C_i^{added} - C_i^{found})^2}{n}} \tag{10}$$

where C_i^{added} is the added concentration of analyte, C_i^{found} is the found concentration of analyte and n is the total number of synthetic mixtures.

Another important parameter is standard error of calibration (SEC) and the calculation of this value was realized by using below equation:

$$SEC = \sqrt{\frac{\sum_{i=1}^{N}(C_i^{added} - C_i^{found})^2}{n - p - 1}} \tag{11}$$

where p is the number of analytes in the samples.

The values of SEP and SEC for CLS and ILS were calculated by using above eqns. 10 and 11, presented in Table-3.

Determination of MAL, EMA, VAN and EVA in commercial products: All the methods were applied to the prepared solutions. Calculated results are presented in Table-4. The calculated values from the statistical test did not exceed the critical statistical values, indicating that there was no significant difference among the methods. The numerical values of all the statistical tests indicated that the elaborated approaches are suitable for the determination of MAL, EMA, VAN and EVA in their commercial samples.

The resolutions of highly overlapping spectra of MAL, EMA, VAN and EVA mixtures were achieved by the use of MLRC, CLS and ILS methods. A selection of working wavelengths having high correlation values with concentration was done during calibration, thus, minimizing variations in concentration due to interference coming from sample matrix and additional analytes outside the working range. MLRC, CLS and ILS methods are suitable choice to methods for the quality control of commercial food product without a priority procedure such as separation, extraction and pre-concentration. The proposed methods have great promise for the routine analysis of food additives.

TABLE-2
RECOVERY RESULTS OF THE SYNTHETIC MIXTURES BY MLRC, CLS AND ILS METHODS

Composition of mixture (mg/mL)				MLRC				CLS				ILS			
				Recovery (%)				Recovery (%)				Recovery (%)			
MAL	EMA	VAN	EVA	MAL	EMA	VAN	EVA	MAL	EMA	VAN	EVA	MAL	EMA	VAN	EVA
2.0	2.0	2.0	2.0	94.1	82.6	87.9	91.9	95.1	84.2	89.6	92.2	96.5	83.2	89.2	92.1
4.0	4.0	2.0	3.0	94.9	95.9	97.8	90.2	93.8	94.1	97.1	89.9	95.5	98.5	96.7	89.9
6.0	6.0	2.0	4.0	81.6	95.6	85.7	84.8	89.9	96.1	86.2	85.9	90.2	97.9	87.3	83.5
8.0	8.0	2.0	5.0	82.9	95.1	91.6	92.5	82.3	97.6	95.8	91.6	84.6	97.3	93.8	96.4
4.0	6.0	4.0	2.0	92.9	95.9	82.4	98.5	90.6	96.1	85.8	99.9	98.1	93.9	89.7	99.6
2.0	8.0	4.0	3.0	86.7	96.4	93.3	92.1	88.4	98.9	93.5	95.4	90.8	98.7	97.9	94.3
8.0	2.0	4.0	4.0	100.5	98.9	100.7	99.3	99.4	104.5	97.5	98.4	104.8	102.0	100.5	95.5
6.0	4.0	4.0	5.0	94.4	92.2	92.8	99.5	95.3	93.9	95.6	100.6	98.4	95.7	92.1	96.6
6.0	8.0	6.0	2.0	102.5	103.1	100.6	96.6	104.9	99.2	101.0	94.9	105.6	99.9	104.5	94.6
8.0	6.0	6.0	3.0	106.9	88.3	89.6	91.3	102.9	87.6	90.2	92.5	101.3	87.3	89.9	102.3
2.0	4.0	6.0	4.0	92.6	94.8	101.8	85.9	95.0	98.1	104.3	88.5	94.3	96.8	99.9	88.3
4.0	2.0	6.0	5.0	103.5	97.9	93.9	84.3	102.6	100.8	92.8	85.6	104.9	100.2	97.5	86.7
8.0	4.0	8.0	2.0	105.9	97.9	90.6	100.4	104.7	99.2	94.0	101.3	106.8	98.9	94.4	102.3
6.0	2.0	8.0	3.0	97.1	98.3	102.9	97.5	97.6	93.8	105.7	92.5	95.9	97.4	100.7	98.9
4.0	8.0	8.0	4.0	98.8	92.1	101.5	101.1	98.3	99.5	102.3	103.1	96.8	95.2	99.7	100.1
2.0	6.0	8.0	5.0	96.6	93.9	98.4	99.5	98.9	94.1	99.9	92.3	98.6	95.2	99.8	95.9
			Mean	95.7	94.9	94.5	94.3	96.2	96.1	95.7	93.4	97.7	96.1	95.9	94.8
			RSD	7.47	4.68	6.29	5.85	6.28	4.96	6.00	6.1	6.1	4.8	5.1	5.5

RSD = relative standard deviation.

TABLE-3
STATISTICAL RESULTS OF THE MIXTURES IN THE CLS AND PCR PREDICTION STEP

Parameters	Methods	MAL	EMA	VAN	EVA
SEP	MLRC	0.5025	0.3310	0.3542	0.3237
	CLS	0.4365	0.2603	0.3196	0.3414
	ILS	0.4066	0.3419	0.2592	0.2956
SEC	MLRC	0.6060	0.3992	0.4272	0.3905
	CLS	0.5264	0.3139	0.3854	0.4118
	ILS	0.4904	0.3285	0.3126	0.3565
r	MLRC	0.9572	0.9902	0.9845	0.9537
	CLS	0.9676	0.9929	0.9860	0.9560
	ILS	0.9695	0.9926	0.9914	0.9659
Intercept	MLRC	-0.1615	-0.0735	-0.2730	0.0830
	CLS	-0.0795	-0.1090	-0.3130	0.0684
	ILS	-0.0975	-0.0475	-0.2100	0.1368
Slope	MLRC	0.9978	0.9665	1.0128	0.9157
	CLS	0.9676	0.9859	1.0342	0.9111
	ILS	1.0023	0.9926	1.0123	0.9040

TABLE-4
EXPERIMENTAL RESULTS OF COMMERCIAL PREPARATIONS OF PROPOSED METHOD

Samples	MAL (μg/g)	EMA (μg/g)	VAN (μg/g)	EVA (μg/g)
Method of MLRC				
A	0.1215	0.1304	0.1632	0.1684
B	0.0592	0.0632	0.2834	0.2950
C	0.1702	0.1158	0.4865	0.3256
D	0.0708	0.0852	0.1140	0.1145
Method of CLS				
A	0.1307	0.1386	0.1902	0.1701
B	0.0606	0.0683	0.2902	0.2964
C	0.1685	0.1252	0.4803	0.3188
D	0.0746	0.0823	0.1203	0.1097
Method of ILS				
A	0.1347	0.1424	0.1926	0.1762
B	0.0616	0.0675	0.2985	0.3031
C	0.1744	0.1202	0.5167	0.3221
D	0.0696	0.0745	0.1194	0.1022

Sample (A) Vanilla. Hayat Food Production Co. Ltd. (B) Candy. Kent Food Production Co. Ltd. (C) Chocolate. Ulker Food Production Co. Ltd. (D) Pudding. Ulker Food Production Co. Ltd. Results obtained are the average of ten experiments for each method.

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