ORIGINAL PAPER

DETERMINATION OF TOTAL PHENOLIC CONTENT FROM PLANT EXTRACTS USED IN COSMETIC PURPOSE

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Abstract. *Spectrometric analysis belongs to a group of measurement methods used for their simplicity and large selectivity to solve various problems of analytes. The total phenolic content of different plant extracts used for cosmetic purpose was determined by a slightly modified version of traditionally Folin-Ciocalteau method. The objective of this work was the validation of UV-Vis spectrometric method, investigation of uncertainty sources when measuring gallic acid concentration and evaluation of the calibration equation effect on measurement uncertainty of UV– Vis spectrophotometer. Validation was performed by studying analytical curve linearity (R^2=0.9995) and range (37.5 – 225 mg L^{-1}), estimated limit of detection (LOD, 0.11mg L^{-1}) and limit of quantification (LOQ, 0.37 mg L^{-1}), precision (%RSD, 0.14 – 1.34), recoveries (83-110%) and stability (%RSD, 0.8 – 2.83). To obtain more representative values for precision, recoveries and stability simultaneous replicates at different times, on different matrices including plant (marigold, chamomile and lavender) were performed during the study period. The validated method was successfully applied to determine TPC in marigold extracts. For chamomile and lavender extracts, the spectrometric method presented only acceptable precision, among all the performance parameters studied. The sources of the gallic acid concentration measurement uncertainty include purity, volume of flasks, mass and the calibration equation. The results indicated that the uncertainty components from purity were the smallest. An important source of the uncertainty was the mass.The volumes of the volumetric flasks had only modest effect on the uncertainty. The contribution of calibration equation is the greatest from all sources.*

Keywords: *total phenolic content; validation; uncertainty; plant extracts.*

1. INTRODUCTION

The environment offers healthy ingredients for natural cosmetics, whereas science and technology helped to better understand their action. Biodiversity loss and climate change can quickly affect the distribution of plants and their chemical ingredients [1-3].

Romania is one of the top six European countries in terms of the number of plant and animal species and is known for its wealth of plants with therapeutic properties, many of which have been used since Thracian times. A great quality appreciated for medicinal species in Romania is that they are not affected by the phenomenon of chemical pollution, and there are still villages where plants are grown as 200 years ago in an unspoiled environment of technology and pollution [4-7].

Plants with therapeutic actions are commonly used, particularly in phytogeographic regions, such as Romania [8]. Marigolds (*Calendula officinalis*) are used in the treatment of

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dry skin, marigold extracts are used in many cosmetics, toiletries and parapharmaceuticals, due to their calming, vasoprotective (improves peripheral circulation, reduces redness), antiseptic, healing and healing effects [9]. Chamomile (*Matricaria chamomilla*) has the most uses in cosmetics, due to its active principles, having anti-inflammatory, decongestant, healing, emollient properties [10]. Chamomile flowers are used mainly as an infusion, for eye, skin and hair care. It is used especially for irritated and congested skin in the form of compresses with 5-10% infusion. Lavender (*Lavandula officinalis*) is used in the treatment of rosacea and acne in the form of compresses, using 5% infusion, it is also used in the care of oily skin [11]. Lavender flowers in bags can also be used for room fragrance. can also be used as a fragrance for clothing cabinets, as well as with other bath plants.

Plant extracts are of great interest in the dermato-cosmetic industry. Today, cosmetic companies explore alternative sources of raw materials, especially plant extracts, because of their valuable source of active substances [12]. Cosmetic companies must use ingredients registered in database created by the European Commission (CosIng) to market their cosmetic products in the European Union [13]. Many countries follow this regulation since their objectives are to export cosmetic products to Europe and to sustain the efficacy of cosmetic ingredients and products [14]. The market value of medicinal plants market is estimated expected to reach about 5 trillion USDby 2050 [15]. A new concept, Cosmetopoeia describes the traditional uses of a plant or a mineral for the embellishment and/or maintenance of the body and is analogous to pharmacopoeia for medicinal plants and purposes [16].

Among the most important characteristics, desired in the case of all cosmetic products are: the moisturizing, inflammatory effect [17], but above all, the antioxidant effect is of major importance and utility [18]. The antioxidant effect is mainly related to the ability of polyphenols contained in plants to annihilate free radicals caused by pollution and solar radiation (main causes of burns, skin cancers, premature aging etc.). The antioxidant capacity of cosmetic products is related also to the content of: carotenoids, tocopherols, ascorbic acid [19, 20].

Phenolic compounds can be classified into several classes, of which phenolic acids, tannins and flavonoids are considered to be the major components of the plant extracts. Structurally, phenolic compounds comprise an aromatic ring that possesses one or more hydroxyl groups and range from simple phenolic molecules to highly polymerized phenolic compounds. Despite this structural diversity, phenolic compounds are often referred to as polyphenols. Polyphenols from plants can positively affect the human body in the treatment of cancer and can be used to treat neurodegenerative diseases [21].

Spectrometric analysis is a widely used measurement method for qualitative and quantitative analysis. The interpretation of a measurement result requires knowledge about its uncertainty. Many important decisions are based on the results of chemical quantitative analysis and it is important to have an idea about the quality of these results [22]. In this regard, several guidelines to evaluate measurement uncertainty in analytical measurements were published [23, 24].

Uncertainty evaluation is not a simple task because of the peculiarities of chemical measurements [25]. The uncertainty of the calibration equation is a source of measurement uncertainty knowing that the establishment of the calibration equation is an essential step for chemical measurements, such as spectrophotometric analysis [26].

Many different methods, including high-performance liquid chromatography (HPLC) in combination with different detectors: UV-Vis, photo diode array (PDA), mass spectrometry (MS), reverse phase-high performance liquid chromatography (RP-HPLC) and UV-Vis spectrometry, have been used to investigate the polyphenolic content in plants [27-32]. However, to the best of our knowledge, no suitable validated UV-Vis spectrophotometric method has been reported for TPC in marigold, chamomile and lavender extracts. Moreover,

the measurement of uncertainty in the TPC analysis was not reported. Analytical Romanian studies have been focused only on estimation of total phenolic content (TPC) from plant extracts by UV-Vis spectrometric method [33-38]. Thus, they could not meet the current technical requirement of ISO:17025-2017 of accurate estimation. The measurement of uncertainty together with method validation, defines the whole quality assurance system by confirming both fitness-for-purpose and reliability of results.

Hence, the objective of the present study is to validate a slightly modified version of traditionally Folin-Ciocalteau method for TPC determination in plant extract for cosmetic purpose. Another objective is to evaluate the uncertainty of measurement results and to evaluate the effect of the calibration equation on measurement uncertainty of UV-Vis spectrophotometer.

2. MATERIALS AND METHODS

2.1. CHEMICALS

Gallic acid was purchased from Fluka (Buchs, Switzerland) and Folin – Ciocalteau reagent from Merck (Darmstadt, Germany). Folin–Ciocalteau reagent was diluted with distilled water 1:2 (v:v). Gallic acid (standard phenolic compound) $2 \cdot 10^{-2}$ mol·L^{-1} was prepared by dissolving 0.376 g of gallic acid in 100 mL ethanol. All other reagents used in the study were of analytical grade procured locally.

2.2. SAMPLE PREPARATION

The plant material (marigold, chamomile and lavender) was collected in the phase of flowering from the natural habitats of the plant in the flowering stage, in the region of the Dobrogea County, Romania, in July 2017.

For the determination of TPC, 10 g plant sample was powdered in a mill and mixed with ethanol 95% (100 mL). Each plant material was mixed with ethanol in brown recipients and left in the dark to obtain the extracts. The mixture was stirred vigurously three times per day. After 3 days the alcoholic extract, previously filtered through paper (Whatman No.1) was collected in glass recipients and stored at 4℃. Each extract was diluted with ethanol in various ratios to prepare the samples for UV-Vis analysis. Ethanol was confirmed to be the best solvent for extracting phenols from marigold flowers [39].

2.3. TPC DETERMINATION

The TPC of plant samples was determined by a slightly modified version of traditionally Folin-Ciocalteau method. The TPC method is based on the reduction of a phosphowolframate – phosphomolibdate complex to blue products by soluble phenolic compounds, in sodium carbonate media and the absorption measurement of the formed complex at 675 nm.

Aliquots of 0.5 mL plant extracts were mixed with 1 mL of 1:2 (v/v) Folin-Ciocalteu reagent in 50 mL calibrated flasks. Then 1 mL ethanol and 1 mL sodium carbonate solution 20% was added. The solution was vigorously mixed, then was allowed to react for 10 min at room temperature and fill up to the mark with distilled water. The resulting mixture were maintained at room temperature for 30 minutes and the absorbance was measured at 675 nm against the corresponding blank, with an UV-VIS spectrophotometer (Jasco 550, Abel Jasco, Germany). Six concentrations of gallic acid (37.5; 75; 112.5; 150; 187.5; 225 mg L^{-1}) were prepared to build the calibration curve. The TPC concentration was calculated from the calibration curve and was expressed as mg gallic acid equivalents per gram of extract (mg GAE/g).

2.4. METHOD VALIDATION

Method validation is the process by which it is established, through a laboratory study, the way in which the performances of the method coincide with the requirements of the analytical applications pursued [38-43].

In this study the analytical method was validated by considering the linear range, limit of detection (LOD), limit of quantification (LOQ), precision, recovery and stability. These parameters must be clearly established in the documentation of the method to their particular needs.

2.4.1. Linearity and range

The linearity of this method was evaluated with standard solutions covering the range between 37.5 and 225 mg L^{-1}. The six solutions with different concentrations of gallic acid were prepared by diluting specific volume of the stock standard solution according to the expected levels in the sample. Triplicate determinations of each calibration standard were done.

The linearity range was tested by homogeneity variance test. To the obtained variances, the *F* test was applied in order to evaluate the significant differences of concentration range limits and to evaluate the regression and lack of fit significances [43]. The calibration is considered suitable if *F* is less than the one-tailed tabulated value (F_{tab}) at a *P* selected confidence level. The *P*-value was determined ($P = \frac{s_{10}^2}{s_1^2}$ for $s_{10}^2 > s_1^2$) [41] and compared with *F* value for n-1 = 9 free degrees to evaluate significance differences.

2.4.2. Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) was the lowest concentration of total phenolic compounds in plant extracts for cosmetic use, that was detectable, but not necessarily quantified, distinguished from zero (signal/noise ≥ 3). The limit of quantification (LOQ) was stated as a concentration below which the method could not operate with an acceptable level of precision and accuracy [40, 41].

Several approaches are possible for determining the LOD and LOQ. Herein, LOD and LOQ were calculated using the calibration data and regression statistic, using the formula:

$$LOD = a + 3\sigma \quad (1)$$

$$LOD = a + 10\sigma \quad (2)$$

where σ is the standard deviation of the response [42, 43].

2.4.3. Precision

Precision of an analytical method is considered at repeatability (intra-day precision) and intermediate precision (inter-day precision). The precision of the developed method was determined by relative standard deviation (% RSD). Two different analysts performed intermediate precision experiments on three different days. For each analyst, the % RSD of ten replicates, was calculated.

Method precision was determined by analyzing results from intra-day and inter-day repeatability of the known concentrations of the standard solutions of gallic acid (n = 10).

To obtain more representative values for the intra-day and between-day intermediate repeatability precision, simultaneous replicates at different times, on different matrices including plant (marigold, chamomile and lavender), were performed during the study period, in the same way as for standard solution [36, 43]. The relative standard deviations are based on the modified Horwitz equation which suggest that RSD<$2^{(1-0,5\,lg\,c)} \times 0.67$, where *c* is the concentration of the analyte.

Repeatability relative standard deviations in the intra-day and inter-day analyses ($\% = 100 \times SD/\bar{x}$) are presented in Table 1.

2.4.4. Recoveries

The accuracy of the proposed method was assessed by performing additions of different concentrations of gallic acid (0.1 mg/mL) to a known pre-analyzed sample (gallic acid of 0.01 mg/L).

Recovery experiments were conducted to determine accuracy of the current method for the quantification of TPC in plant extracts for cosmetic use. The recovery studies were carried out in triplicate for replicates of the gallic acid analyte concentration (0.01 mg/L), marigold, chamomile and lavender samples. The % recovery of the added gallic acid was calculated as [40, 41]:

$$\%recovery = \frac{C_t - C_s}{C_a} \times 100 \quad (3)$$

where *C*t is the total analyte concentration measured after standard addition; *C*s, analyte concentration in the sample; *C*a, analyte concentration added to sample.

2.4.5. Stability

The stability of the gallic acid standard solution was evaluated as well as the stability of marigold extract during a day, by keeping samples in 50 mL volumetric flask at room temperature (performing 12 readings at every half hour).

2.4.6. Measurement of uncertainty

In this study were evaluated the uncertainty sources of the gallic acid measurement with the UV-Vis spectrophotometer. The uncertainty measurement characterizes the

dispersion of the values that could reasonably attribute to the content of targeted analytes [43]. All possible uncertainty sources were taken into account.

The concentration of gallic acid (mg/L) was calculated as follows [43]:

$$C_{gallic\ acid} = \frac{m \times 100 \times P}{V} \quad (4)$$

where m is the mass of gallic acid, P is the purity of gallic acid, V is the volume of the 100 mL volumetric flask and 1000 is a conversion factor from mL to L.

Purity of gallic acid

The purity for the standard substance is shown on the producer certificate. The purity was assumed to be the uniform distribution [24].

Mass

The uncertainty sources of the weighting included resolution of balance, repeatability uncertainty of weight and uncertainty of balance scale calibration function. This function has two potential sources: the sensitivity of the balance and its linearity. The sensitivity can be neglected because the weighing is performed on the same balance over a very narrow range. The standard uncertainty of resolutions and calibration weights were assumed as the uniform distribution [24]. The standard uncertainty of repeatability was assumed as the normal distribution.

Volume

The volume of the solution contained in the 100 mL volumetric flask is affected by three major sources of uncertainty. They were the calibration uncertainty of the flask volume, uncertainty of replication of the flask volumes and uncertainty of the volume caused by difference in temperature between the basic temperature for the flask and the temperature of laboratory. The calibration uncertainty of the flask volume was assumed as the normal distribution. The other sources of uncertainty were considered as the uniform distribution [24].

The calibration equation

Instrument calibration is an essential stage in most measurement procedures. It is a set of operations that establish the relationship between the output of the measurement system (*e.g.*, the response of an instrument) and the accepted values of the calibration standards (*e.g.*, the amount of analyte present).

In relation to instrument calibration, the aim of linear regression is to establish the equation that best describes the linear relationship between instrument response (y) and analyte level (x).

The uncertainty source of the calibration equation is assumed to originate from the variability in dependent value. The linear calibration equation is: $y = a + bx$, where y is the absorbance of the UV–Vis spectrophotometer, x is the concentration of gallic acid standard solution and a and b are the coefficients of the calibration equation.

It is possible to calculate a confidence interval for values predicted using the calibration function. This is sometimes referred to as the ‘standard error of prediction’. The prediction interval gives an estimate of the uncertainty associated with predicted values of x.

The residual standard deviation (also known as the residual standard error) is a statistical measure of the deviation of the data from the fitted regression line. It is calculated using Eq. 2:

$$s(r) = \sqrt{\frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{n-2}} \quad (5)$$

where y_i is the observed value of y for a given value of x_i, $\hat{y}$ is the value of y predicted by the equation of the calibration line for a given value of x_i, and n is the number of calibration points.

The prediction interval s_{x_0} is calculated using Eq. 3:

$$s_{x_0} = \frac{s(r)}{m} \times \sqrt{\frac{1}{N} + \frac{1}{n} + \frac{(\overline{y_0} - \overline{y})^2}{b^2 \sum_{i=1}^{n}(x_i - \overline{x})^2}} \quad (6)$$

where:

s(r) is the residual standard deviation (see Eq. 5)
n is the number of paired calibration points (x_i, y_i)
m is the calculated best-fit gradient of the calibration curve
N is the number of repeat measurements made on the sample
$\overline{y_0}$ is the mean of N repeat measurements of y for the sample
$\overline{y}$ is the mean of the y values for the calibration standards
x_i is a value on the x-axis
$\overline{x}$ is the mean of the x_i values
The predicted value x_{pred} was calculated from the measured $\overline{y_0}$value.

$$x_{pred} = \frac{\overline{y_0} - a}{b} \quad (7)$$

3. RESULTS AND DISCUSSION

3.1. METHOD VALIDATION

3.1.1. Linearity and range

The test of homogeneity variance established a P = 1.20 and the following acceptance criterion: P < F_{tab} value (5.35 for n-1=9 free degrees). This means that no significant differences were found between the variances of the concentration range limits.

The above results showed that a linear calibration for the analytical method over the calibration ranges tested (37.5 to 225 mg L^{-1}) was obtained. Determination coefficients (R^2) obtained from linear regression analysis were 0.9955. Equation of the calibration curve for gallic acid is y = 0,0041x.

This correlation coefficient is comparable to that obtained using other methods listed in the references, e.g. 0.9985 (for the method in [46]) and 0.996 (for the method in [47]).

3.1.2. Limit of detection (LOD) and limit of quantification (LOQ)

Method LOD and LOQ, calculated using calibration data and regression statistics from gallic acid calibration curve, were found to be 0.11 mg L^{-1}, respectively 0.37 mg L^{-1}. Thus, the present method is able to quantify compounds with similar reactivity in reaction with Folin Ciocalteau reageant, like TPC even at low concentrations.

3.1.3. Precision

Precision determines effect of random errors on repeatability of the method which is expressed as % RSD. The obtained % RSD values for the standard solution of gallic acid, for marigold, chamomile and green tea samples, were lower than those obtained using the Horwitz equation (Table 1).

The precision was successfully demonstrated by achieving % RSD of 0.10 – 5.66 % for replicate determinations of standard solution and real samples in the intra-day precision experiment. The % RSD values for precision studies with real samples of plants (marigold, chamomile and lavender) were found to be less than 6.22 %. In the intermediate precision experiment, RSD values results indicating no considerable difference between the experiments irrespective of the analyst and day of sample analysis, under otherwise identical conditions (Table I).

The overall % RSD for intra-day and inter-day precision for the presented method were found to be less than 2 % for marigold and chamomile, considering % RSD less than 2 % to be acceptable.

The relatively low % RSD observed indicates the method has good precision enabling quantification of TPC in plant extracts.

Table 1. Precision study of the proposed method

Samples	***Intra-day precision RSD% (n=10)***	***Inter-day precision RSD% (n=10)***					
		Day 1		***Day 2***		***Day 3***	
		Analyst 1	Analyst 2	Analyst 1	Analyst 2	Analyst 1	Analyst 2
Standard solution of gallic acid	0.83	0.28	0.10	0.09	0.12	0.81	0.22
Marigold	1.34	0.14	0.43	0.11	0.09	1.50	1.30
Chamomile	0.22	1.30	1.22	0.38	0.57	0.67	0.29
Lavander	5.66	6.22	5.39	2.60	2.00	0.22	0.35

3.1.4. Recoveries

The recovery values obtained were between 50 -70 % for lavender, 70-120 % for chamomile and 83 -110 % for gallic acid, respectively marigold. The acceptance criterion for fortification tests requires recovery values within the range of 80 to 110 % (limits imposed by Horwitz function) [44]. These percentage recoveries showed that proposed method is accurate for gallic acid and marigold.

3.1.5. Stability

The standard solution of gallic acid and marigold extract was used to investigate possible changes over time. The % RSD values for the standard solution of gallic acid and for

marigold were 2.83 % respectively 0.8 %. The method proved the stability of both solutions during a day.

3.1.6. Quantifying the uncertainty components

To meet the requirement of reliability criteria of analysis, several uncertainties measurement data were generated.

Purity of gallic acid

The purity of gallic acid as shown on the manufacture certificate was 0,9999 ± 0,0001. The standard uncertainty is calculated by assuming it as a rectangular distribution.

$$u(P) = \frac{0{,}0001}{\sqrt{3}} = 0{,}00006 \quad (8)$$

Mass

The uncertainty associated with the gallic acid mass is 0.05 mg and is assessed using the data calibration certificate and the manufacture's recommendations.

Volume

a. The manufacture's calibration of flask volumes

The specification of flask volume from manufacture is quoted the volume 100 mL ± 0.1 mL. The standard uncertainty is calculated by assuming it as a triangular distribution.

$$\frac{0{,}1\ \text{mL}}{\sqrt{6}} = 0{,}04 \quad (9)$$

b. Repeatability of flask volumes

The calibration of flask volumes was executed by filling water into flask to the height of 100 mL marked at the flask. The weight of the water then be measured by balanced. There were 10 such measurements that highlighted a standard uncertainty 0f 0.02 mL.

c. Temperature

According to the specification of the manufacture, the flask was calibrated at 20 °C. The laboratory temperature varied between the limits of ± 4 °C. The volume expansion coefficient for water is 2,1 x 10^{-4} $°C^{-1}$. The volume variation in this study was ± (100 mLx 4x2,1x10 $^{-4}$) = ±0,084 mL. The standard uncertainty is calculated by assuming it as a rectangular distribution.

$$\frac{0{,}084\ \text{mL}}{\sqrt{3}} = 0{,}0485\ \text{mL} \quad (10)$$

The uncertainty of the temperature is:

$$u(V) = \sqrt{u(V_{cal}) + u(V_{rep}) + u(V_{temp})} = 0{,}07\ \text{mL} \quad (11)$$

The uncertainty of the calibration equation

The relationship between the absorbance of UV–Vis spectrophotometer and the gallic acid concentration of the standard solution is presented in Fig. 1. The linear inverse equation is y = 0,0041x; R^2 = 0.9955 where y is the gallic acid concentration in mg/mL, x is the absorbance of UV–Vis spectrophotometer and R^2 is the coefficient of determination. Table 2 shows the data required to calculate the prediction interval.

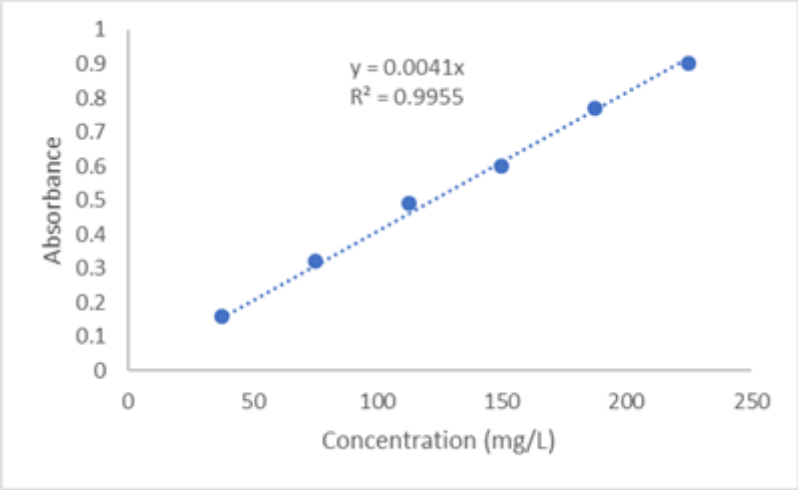

Figure 1. The calibration curve for the determination of gallic acid.

Table 2. Data required to calculate the prediction interval

Concentration x_i n=8	**Absorbance** y_i	$(x_i - \bar{x})^2$	**Predictable values** $\hat{y}_i = bx_i + a$	**Residuals values** $y_i - \hat{y}_i$	**Residuals values^2** $(y_i - \hat{y}_i)^2$
37.5	0.160	7931.68	0.153	0.007	0.000049
75	0,320	2658.43	0.307	0.013	0.000169
112.5	0,490	197.68	0.461	0.029	0.000841
112.5	0.388	197.68	0.461	-0.073	0.005329
112.5	0.490	197.68	0.461	0.029	0.000841
150	0,600	549.43	0.615	-0.015	0.000225
187.5	0,770	3713.68	0.768	0.002	0.0004
225	0,900	9702.25	0.922	-0.022	0.000484
$\bar{x}$	$\bar{y}$	$\sum_{i=1}^{n}(x_i - \bar{x})^2$			$\sum_{i=1}^{n}(y_i - \hat{y}_i)^2$
126.56	0.514	3143.56			0.008338

Using Eq. (2) the residual standard deviation is calculated as:

$$s(r) = \sqrt{\frac{0.008338}{8-2}} = 0{,}037 \quad (12)$$

Applying Eq. (3), the prediction interval is:

$$s_{x_0} = 11.08\ mg/L \quad (13)$$

$$x_{\text{pred}} = \frac{0.5343-0}{0.0041} = 130.31\ mg/L \quad (14)$$

Expressed as a % of x_{pred}, $s_{x_0} = 8.50\%$.

At the 95% confidence level, the 2-tailed Student t value for 5 degrees of freedom is 2.571. The 95% confidence interval for x_{pred} is 0.037 x 2.571 = 0.095 mg L^{-1}.

Uncertainty contributions in gallic acid measurement by UV–VIS spectrophotometer

From the various source's uncertainty listed in Table 3, the uncertainty components from purity were the smallest. The volumes of the volumetric flasks had only modest effect on the uncertainty. The mass was an important source of the uncertainty. The contribution of calibration equation is the greatest from all sources.

Table 3. Values and uncertainty in gallic acid measurement by UV–Vis spectrophotometer

Description	Value of calculated uncertainty	Standard uncertainty u(x)	Relative standard uncertainty u(x)/x
Purity, P	0.9999	0.00006	0.00006
Mass, m (mg)	376 mg	0.05	0.0001329
Volume,V (mL)	100 mL	0.07	0.0007
Calibration	11.08	8.5	0.76

Global uncertainty was calculated by the following formula:

$$\frac{u_c(c_{\ gallic\ acid})}{c_{\ gallic\ acid}} = \sqrt{(\frac{u(P)}{P})^2 + (\frac{u(m)}{m})^2 + (\frac{u(V)}{V})^2 + (\frac{u(c.c)}{c.c})^2} = 0{,}76 \quad (15)$$

$$u_c(c_{\ gallic\ acid}) = c_{\ gallic\ acid} \times 0{,}76 = 0.285\ mg/L \quad (16)$$

The expanded uncertainty was calculated by multiplying global uncertainty by a coverage factor k = 2, for defining the results of unknown true value with a confidence level of 95%.

3.2. METHOD APPLICABILITY

Phenolic compounds are commonly found in plants and they have been reported to have multiple biological effects, including antioxidant activity. Phenolic contents such as phenolic acids and anthocyanins contributed towards the strong antioxidant capacities of marigold flowers [48]. The phytochemical composition of edible flowers, with the common name marigold, revealed them to be wonderful natural gifts containing many therapeutic values [8].

The validated method was used to quantify the TPC in marigold extracts. The amount of TPC varied in the studied marigold flower extracts, ranging from 29.85 to 62.51 mg GAE/g extract. Many studies reported that TPC varied considerably in marigold cultivars [45, 48] and among edible flowers [49]. The results obtained in this study are comparable with those reported by Ingkasupart et al and Velicković et al [49, 50].

4. CONCLUSIONS

To the best of our knowledge, s this validated UV–vis spectrophotometric assay based on a modified Folin-Ciocalteau method has been applied for the first time to measure TPC in marigold, chamomile and lavender extracts used in cosmetic purpose. Additionally, uncertainty measurement was investigated to allows the correct interpretation of the analytical results.

The slightly modified version of traditionally Folin-Ciocalteau method was successfully applied to the determination of TPC in marigold extracts. Present method shows acceptable and satisfactory performance for all the tested validated parameters for marigold extracts. In case of chamomile and lavender extracts, the spectrometric method presented only acceptable precision.

To meet the quality goals in analysis were evaluated the uncertainty sources of the gallic acid measurement. From the uncertainties measurement data were observed that the effect of calibration equation has the greatest impact on uncertainty of spectrometric analysis results.

In conclusion the results of the study demonstrate that the accuracy and precision evaluation of the present method was quite satisfactory for TPC determination in marigold extracts used in cosmetic purpose. Compared to other available methods, the technique is advantageous in that it is simple, precise, accurate and inexpensive.

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