Determination the Total Phenolic Contents in Some Foods Using Solid Phase Extraction

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Abstract

Solid phase extraction with Amberlite XAD-8 resin was used for the determination of total phenolic contents in some foods using Folin-Ciocaltue's method and formation blue complex absorbance measured at λ max 765 nm. The effecting factors pH, time of extraction, number of the extraction batches and elution solvent was studied. The obtain results showed that the highest extraction occurs at optimal conditions, pH=2 at extraction time 10min. and using acetonitrile as elution solvent. The distribution coefficient K_d , for the adsorption and elution has been calculated for Gallic acid, 7-hydroxy coumarin. This resin prove to be capable for adsorption of these compound from aqueous samples, and acetonitrile was the supreme solvent for elution owing to superior polarity in addition to dielectric constant compare with ethanol and diethyl ether. The precision and accuracy of this method was found by analysis a laboratory made of Gallic acid samples, the statistical managements showed relative error ranged from ± 0.842 to 9.462%, mean error equal to -2.440, standard deviation 1.149, relative standard deviation not exceed 2.416%, and confidence limit 47.560±1.426. The effectively applied result of this method for determination of total phenolic contents in eight different type of food samples showed that the amount was varying from the highest value in dates which equal 10.466 mg GAE/10g sample and 1.160 mg GAE/10g sample in kiwi as lowest value.

Keywords : Total phenolic content; Folin-Ciocalteu reagent; Amberlite XAD-8; Foods.

Introduction

Phenolic compounds widely distributed in the medicinal plants, spices, vegetables, fruits, grains, pulses and other seeds are an important group of natural antioxidants with possible beneficial effects on human health. They can participate in protection against the harmful action of reactive oxygen species, mainly oxygen free radicals. Free radicals are produced in higher amounts in a lot of pathological conditions and are involved in the development of the most common chronic degenerative diseases, such as cardiovascular disease and cancer [1,2].

Phenolic compound were extracted by many methods like, liquid-liquid extraction and solid phase extraction [3-6].

Solid Phase Extraction, SPE was developed in early to mid-1970s, solid particles such us activated carbon, silica gel and porous polymeric resins known as sorbent. SPE is rapid, easy and economical alternative to solvent extraction. It was used to extraction compounds from a liquid matrix or as a complement to solvent extraction.In SPE, solutes are extracted from a liquid into solid phase. The extraction can occur in a batches method in which the solid extract is intimately mixed with the liquid sample solution or column by packed the solid extract into a small tube and pass the liquid sample through the tube [7]. SPE used for the extraction and separation of dissolved organic (DOM) has not been investigated much although the extraction of fuel oil and synthetic organic material has been studied [8]

Amberlite XAD resins are available with a variety of polarities, XAD-1, XAD-2, XAD-4, XAD-7 and XAD-8, the potential of these resins for the extraction and separation of organic compounds from complex aquatic different samples has been studied. The adsorptive forces present when using amberlite XAD resins as adsorbent are primarily of the Van deer walls type [9,10]. Amberlite XAD-2 (pore size 9mm, particle size 0.3–1.2 mm) was used for isolation of phenolic compound from Australian honey [11].

The purpose of this study was find a simple method for the determination of total

phenolic contents in different type of foods employing Amberlite XAD-8 resin and Folin-Ciocalteu's phenol reagent.

Experimental Part

Chemicals and instruments:

A LABOMED-UVD-2960 UV-Vis double beam spectrophotometer equipped with PC and 1 cm quartz cell were used to measure the absorbance at λ_{max} of each phenols analyte, WTW IMOLAB Terminal 140 pH meater.

All compounds, reagent and solvents were at high purity obtained from commercial sources and no further purification was need and used as received. Amberlite XAD-8 resin produced by Fluka with a 20-50 mesh number, hydrochloric acid, ethanol, acetonitrile and diethyl ether were obtained from BDH company, sodium hydroxide from Rasayane company and gallic acid, 7-hydroxy coumarin and Folin-Ciocaltue's reagent were obtained from SIGMA company.

Condition of Amberlite XAD-8 resin Amberlite XAD-8 resin was used after cleaning to remove monomers and soluble uncross-linked polymer. The resin washed and cleaning using soxhlate for 24h with acetonitrile, Then washing with vigorous shaking with 0.1 N NaOH solution, finally washed with de-ionized water, the purity of the resin was cheeked in blank procedure and the purified resin stored under distilled water.

Samples preparation and digestion

The food samples were washed many time with distilled water and left to dry for 1h. Three procedure were used to prepare each sample for SPE with Amberlite XAD-8 resin. The first method: an accurate 5 grams of sample was chopped in to small pieces, then add 25ml aqueous solution the pH was adjusted to 2 used pH meter by dropping HCl, heating at 60 ºC with stirring for 1 h. then allow to stand in dark place for 24 h. The second procedure: was similar to the first one except no heating was used when stirring the sample. The third procedure involve using 25ml of 80% ethanol solution. finally in all these procedures, The solutions were filtered during Whatman filter paper.

Solid Phase Extraction of phenolic compounds

To optimization the extraction method the effect of pH, extraction time and elution of extracted phenolic compoundwere studied used gallic acid and 7-hydroxy coumarin as standard materials each compound were studied alone.

pH effect

The effect of sorbent resin and pH optimization were carry through concurrently. Four different values of pH were studied, 1, 2, 3 and 4 the pH was adjusted by pH meter by dropping HCl.

Elution of extracted phenolic compounds

The upper aqueous layer was exactly removed, then the resin was washed several time with distilled water to remove and clean any impurities. To elute or desorption the adsorption phenolic compounds from the resin, a 4ml of suitable solvent, acetonitrile, ethanol and diethyl ether was added and shake vigorously for 10 min.

Standard calibration graph

A standard calibration curve for Gallic acid in the concentration extent 25-125 $mg.L^{-1}$ were prepared by dilution (0.5,1,1.5,2,2.5 ml) from stock standard solution 1000 $mg.L^{-1}$ to 20 ml. The regression equation [$Y = Xb \pm a$, where b is the slope=0.0046, a is the intercept=-0.0059, X is the concentration and Y is the absorbance] was utilized by Least Square method [12].The propriety of the regression equation was tested by analyzing laboratory samples. Beers law is sensible within the concentration ranges of calibration graph.

Food sample extraction

In a suitable volume quick fit conical flask, the aqueous of digestion solution was added to a 5 g of cleaned XAD-8 resin and shake vigorously for 10 min.

Determination of total phenolic contents

The total phenolic contents in the food samples extracts was estimated by Folin–Ciocalteau phenol reagent assay described by Singleton and Rossi with minor modifications [13]. A 0.12 ml of eachstandard sample

(25,50,75,100,125 mg.L^{-1}) or eluted sample or blank solution transfer to a suitable test tube and combined with 0.15 ml of Folin–Ciocalteus reagent. The sample were shake for 15 sec and then allowed to stand for 6 min., then 0.45 ml of 20% aqueous sodium carbonate solution was added, and the mixture was diluted to 3 ml by adding 2.28 ml de-ionized water, finally heated at 45ºC for 30min. The absorbance was measured at 765 nm using UV–Vis spectrophotometer. The amount of total phenolic contents was represented as Gallic acid equivalent (GAE) in milligram per 10gram fresh sample, using standard calibration graph [14] .

Statistical analysis

All data-processing were set in a wholly randomized design with three to ten replicates. Data were analyzed by SPSS computer software version 20.

Result and Discussion

Extraction yields

A solid phase extraction was used for the determination of total phenolic contents from different type of foods. The extraction yield affecting with many factors include: pH of aqueous sample, the type and polarities of the elution solvents, extraction time, number of batch extraction and the chemical compositions of the sample[15, 16] the extraction percent were calculated from the equation below

$$\text{Extraction \%} = \frac{\text{Initial conc.} - \text{Final conc.}}{\text{Initial conc.}} * 100$$

pH effect

A given acid will lose a proton in an aqueous solution depends on the pK_a of the acid and the pH of the solution. The relationship between the two parameter is given by the Henderson–Hasselbalchequation [17]. This is an extremely useful equation because it tells us whether a compound will exist, in its acidic form, with its proton retained or in its basic form, with its proton removed at a particular pH .

The results shows that the effect of pH on Gallic acid extraction was *Significance*with R^2 which equals to -0.95 while for 7-hydroxy coumarin, the pH effect was not *Significance*, Fig.(1).

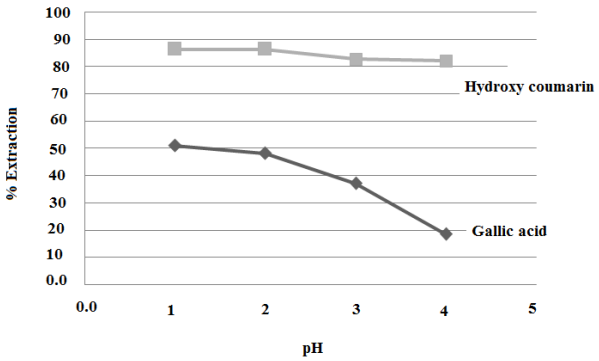

Fig.(1) Effect of pH for SPE of Gallic acid and hydroxy Coumarinat 5 mg/L concentration and extraction time 10 min.

Extraction time effect

Extraction time is one of the most important factor in SPE analysis, which it allow the analyte to reach equilibrium with the resin. The extraction time of these compounds ranges from a few minutes to a few hours, the short time and high value of distribution constant K_d means that the separation method is better [18]. Results showed that the optimize time for extraction of phenolic compounds is 10 minutes , while the 15 minutes gave a little increase in the amount which is negligible and not important. The increase in extraction time undesirable, which it may be oxidation phenolic compounds as a result of exposure to light and oxygen when increased time of extraction [19]. Also the result show that the extraction time for coumarin is less than Gallic acid, Fig.(2).

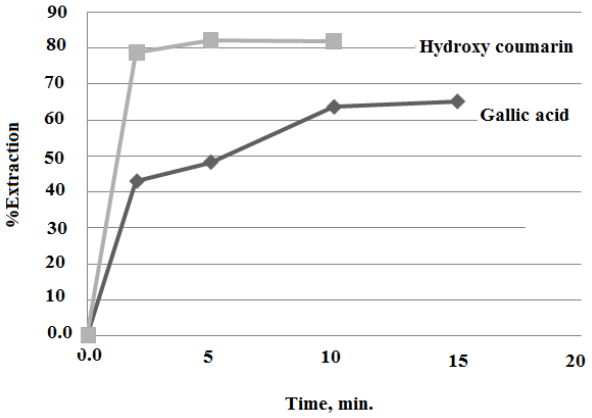

Fig.(2) Effect of extraction time for Gallic acid and hydroxy coumarin at 5 mg/L concentration and pH=2.

Elution

To eluted and recovery the adsorbed phenolic compounds, three organic solvent acetonitrile, ethanol and diethyl ether were used which differ in physical and chemical properties, Table (1), the elution percentage calculated from the equation below:

$$\text{Elution \%} = \frac{\text{conc.of sample in organic solvent}}{\text{conc.of sample uptake by the resin}} * 100$$

The highest elution at different time were obtained with acetonitrile followed by ethanol then diethyl ether, Fig.(3, 4).

Table (1)
Physical and chemical properties of organic solvent used.

solvent	*Density g/cm^3*	*Viscosity cP*	*Dielectric constant, ε0*	*Dipole moment, D*	*polarity*
Ethanol	0.79	1.10	24.55	1.96	8.80
Acetonitrile	0.78	0.38	37.50	3.92	18.00
Diethyl ether	0.71	0.24	4.30	1.15	2.90

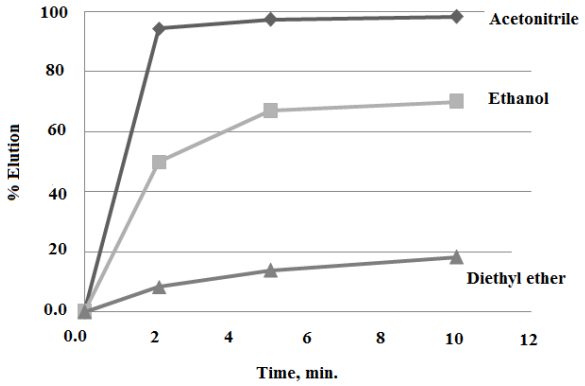

Fig. (3) Effect of solvents for Gallic acid elution at different extraction time.

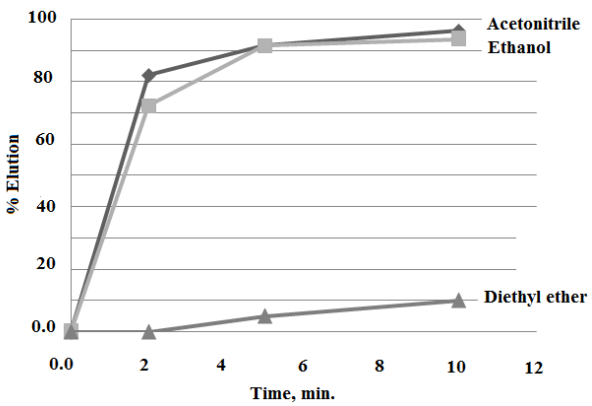

Fig. (4) Effect of solvents for hydroxy coumarin elution at different extraction time.

Analysis

A standard calibration curve for Gallic acid Fig.(5), in the concentration extend 25-125 mg.L^{-1} were used for calculation the total phenolic contents concentration as Gallic acid equivalent. The accuracy and precision of the analysis was examine. The statistical analysis for ten laboratory sample from Gallic acid 50 mg.L^{-1} were prepared by dilution 1.25 ml from stock solution 1000 mg.L^{-1} to 25 ml, showed relative error ranged from ± 0.842 to 9.462%, mean error equal to -2.440, standard deviation 1.149, relative standard deviation not exceed 2.416%, and confidence limit 47.560±1.426, Table (2).

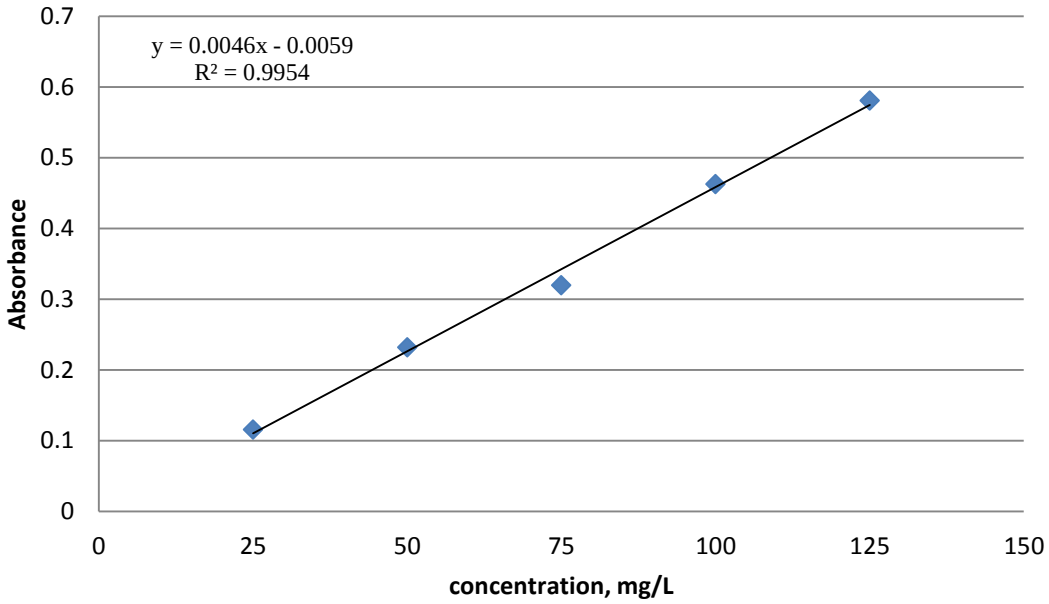

Fig.(5) Calibration curve for Gallic acid at concentration ranged from 25-125 mg/L at 765nm.

Table (2)
Statistical analysis for ten laboratory Gallic acid samples (50 mg.L^{-1}).

Sample No.	*Conc. Found in mg.L^{-1}*	*Recovery*	*Absolute error*	*Relative Absolute error %*	*Mean error*	*Standard deviation*	*RSD %*	*Conf. limit at 95%*
1	47.424	94.848	-2.576	-5.152	-2.440	1.149	2.416	47.560±1.426
2	46.562	93.124	-3.438	-6.876				
3	45.269	90.538	-4.731	-9.462				
4	47.855	95.710	-2.145	-4.290				
5	47.627	95.254	-2.377	-4.754				
6	48.241	96.482	-1.759	-3.518				
7	47.158	94.316	-2.842	-5.684				
8	47.424	94.848	-2.576	-5.152				
9	48.452	96.904	-1.548	-3.096				
10	49.579	99.158	-0.421	-0.842				

Total phenolic content

Sample were determined using regression equation of calibration graph (b=0.0046, R^2=0.9954) and expressed in Gallic acid equivalents, Total phenolic content express in mg/10 g by using the equation below:

Conc. mg GAE/10 g sample= conc. mg.L^{-1} * 0.004 L*2.

The analysis results showed that their values varied between 10.446±0.482 mg/10 g

sample for dates as highest valueand 1.16±0.107 mg/10 g sample for Kiwi fruit as the lowest value, Fig. 6, these values represent the mean ± SD.

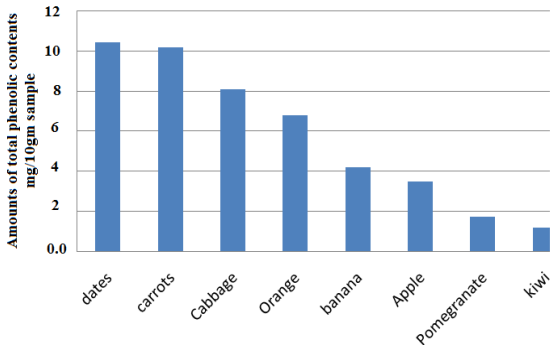

Fig. (6) Total phenolic contents as mg/10 g fresh sample find in eight food samples.

Linearity, LOD and LOQ

Under the optimized UV-VIS conditions with linear regression analysis, y = 0.0046x – 0.0059, for the concentration range 25-125 mg/L, with R^2 0.9954, LOD varied from 0.571 mg/10g sample for apple to 1.990 for banana and LOQ ranged from 1.884 mg/10g sample for apple and 6.567for banana, Table (3).

Table (3)
Total phenolic quantities find in food sample ± mean SD.

binomial nomenclature	*Food sample*	*Concentration mg GAE/10g sample*	*LOQ mg*	*LOD mg*
Malusdomestica	Apple	3.491 ± 0.173	1.884	0.571
Actinidiadeliciosa	Kiwi	1.160 ± 0.107	1.165	0.351
Musa Paradisiaca	Banana	4.176 ± 0.603	6.567	1.990
Punicagranatum	Pomegranate	1.727 ± 0.216	2.352	0.713
DaucusCarota	Carrots	10.200 ± 0.346	3.768	1.142
Palmaedactylifera	Dates	10.446 ± 0.482	5.249	1.591
Brassica oleracea	Cabbage	8.080 ± 0.264	2.875	0.871
Citrus sinensis	Orange	6.804 ± 0.384	4.182	1.267

Conclusion

The results showed that the competence of extraction achieve when occur at pH=2 for aqueous sample and extraction time 10 min. with two batches, this gave a high extraction percent not lower than 96.41%, this means that the repetition of the extraction process two times increase the aptitude the extraction, and the acetonitrile was the more suitable solvent for elution due to powerful polarity of solvent as well as dielectric constant compared with ethanol and diethyl ether.

Reference

[1] Block G., Patterson B., SubarA., “Fruit, vegetables, and *cancer* prevention: a review of the epidemiological evidence”, *Nutr. Cancer* 18,1, 1-29, 1992.

[2] Halliwell B., Gutteridge J.M.C., “Free Radical in Biology and Medicine”, Oxford University Press, UK, pp. 617, 1999.

[3] Hua-Bin L., Ka-Wing C., Chi-Chum W., King-Wai F., Feng C., Yue J., "Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae", *Food Chemistry* 102, 771–776, 2007.

[4] Nihal T., Ferda S., Y. SedatV., "Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin–Ciocalteu methods", *Food Chemistry* 99, 835–841, 2006.

[5] Ratiporn H., Sumitra P., Suchada V., Jacek N., Magda S., Yong-Seo P., Buk-Gu H., Ja-Yong C., Hong G., Shela G., "Comparison of bioactive compounds, antioxidant and antiproliferative activities of Mon Thong durian during ripening", *Food Chemistry* 118, 540–547, 2009.

[6] Abdelhak M., Guendez E., Eugene K., Panagiotis K., "Phenolic profile and antioxidant activity of the Algerian ripe date palm fruit (Phoenix dactylifera)", *Food Chemistry* 89, 411–420, 2005.

[7] James S. Fritz, "Analytical Solid-Phase Extraction", John Wiley and Sons, New York, 1999.

[8] Mills, G.L., Quinn, J.G., "Isolation of dissolved organic matter and copper organic complexes from estuarine waters using reverse phase liquid chromatography". *Marine Chemistry* 10, 93-102, 1981.

[9] PietrykD. J., "Ion-exchange resins in non-aqueous solvents-III Solvent-uptake properties of ion-exchange resins and related adsorbents". *Talanta* 16, 169, 1969.

[10] Wilks A. D., Pietrzyk D.J., "Heats of immersion and swelling of cation resins and related model systems in water and nonaqueous solvents". *Anal. Chem*. 44, 676, 1972.

[11] Izabela J., Anna P., Małgorzata D., Paweł K., "Phenolic compounds and abscisic acid as potential markers for the floral origin of two Polish unifloral honeys", *Food Chemistry* 131, 1149–1156,2012.

[12] James N. Millar & Jane C. Millar,. "Statistic and Chemometrics for analytical chemistry", 4th Ed, Prentice Hall, 2000.

[13] Singleton, V. L., & Rossi, J. A. J., "Colorimetric of total phenolics with phosphomolybdic - phosphotungstic acid reagents". *American Journal of Enology and Viticulture* 16, 144–158, 1965.

[14] Rosana C., Jorge G., Indira B., Romina P., David C., "Antioxidant compounds and antioxidant capacity of Peruvian camucamu (Myrciariadubia (H.B.K.) McVaugh) fruit at different maturity stages", *Food Chemistry* 120, 1019–1024, 2010.

[15] Ganesan, P., Kumar, C. S., & Bhaskar, N., "Antioxidant properties of methanol extract and its solvent fractions obtained from selected Indian red seaweeds". *Bioresource Technology*, 99, 2717–2723, 2008.

[16] Yuan, V. Y., &Walsh, N. A., "Antioxidant and proliferative activities of extractsfrom a variety of edible seaweeds". *Food and Chemical Toxicology* 44, 1144–1150, 2006.

[17] Lapornik B., Prosek M., Wondra A.G., "Comparsion of extracts prepared from plant by products using different solvents and extraction time". *Journal of Food Engineering* 71, 214 – 222, 2005.

[18] Catherine N, Gerard S., Edmond R., Pierre A., Christian R., "Genetic Variability Influences Carotenoid, Vitamin, phenolic and mineral content in white, yellow, purple , orange and dark orange Carrot cultivars". *J. Amer. Soc. Hort. Sci* 129(4), 523-529, 2004.

[19] Silva E.M., Rogez H., Larondella Y., "Optimization of extraction of phenolics from Inga edulis leaves using response surface methodology". *Separation and Purification Technology*, 55, 381-387, 2007.

الخلاصة

استخدمت طريقة الاستخلاص بالطور الصلب باستعمال الراتنج Amberlite XAD-8 لتقدير المحتوى الفينولي الكلي في بعض الاغذية و باستخدام كاشف Folin-Ciocaltue's و تكوين معقد ازرق اللون و قياس الامتصاصية عند اعظم طوال موجي 765 نانومتر، تم دراسة المتغيرات التالية: الدالة الحامضية، زمن الاستخلاص، عدد وجبات الاستخلاص و المذيب الافضل لعملية الابتزاز و اظهرت النتائج ان افضل نسبة للاستخلاص عند الظروف المثلى دالة حامضية 2 و عند زمن استخلاص 10 دقائق مع استخدام مذيب نتريل المثيل كافضل مذيب لاسترجاع المركبات الفينولية من الراتنج، تم حساب معامل التوزيع K_d لكل من عمليتي الامتزاز و الابتزاز للمركبات حامض الكاليك و ٧-هيدروكسي كومارين، اظهر الراتنج قدرة على امتزاز هذه المركبات من المحاليل الحامضية و ان مذيب نتريل المثيل افضل مذيب لابتزاز هذه المركبات من الراتنج لامتلاكه افضل قطبية و عزم ثنائي القطب بالمقارنة مع الايثانول و ثنائي اثيل ايثر، تم دراسة الدقة و الضبط للطريقة قيد الدراسة بتحضير عينات تخليقية مختبرية من حامض الكاليك و اظهرت المعالجات الاحصائية خطأ نسبي يتراوح بين 0.842- الى 9.426 %- و خطأ وسطي يساوي ٢.٤٤٠- وانحراف قياسي 1.149 و انحراف قياسي نسبي مئوي 2.416% و كانت حدود الثقة 47.560±1.161، استخدمت النتائج التطبيقية لهذه الطريقة لتقدير المحتوى الفينولي الكلي في ثمانية انواع مختلفة من الاغذية و كانت النتائج متباينة من اعلى قيمة في التمر و تساوي 10.466 ملغم مكافئ حامض الكاليك/١٠ غرام عينة و 1.160 ملغم مكافئ حامض الكاليك/١٠ غرام عينة فاكهة الكيوي كاقل قيمة.