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Research Article

ANALYSIS OF ELLAGIC ACID IN FRESH AND PROCESSED FRUIT PRODUCTS BY HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

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ABSTRACT
Ellagic acid, a dimeric compound of Gallic acid is found in various fruits and vegetables. They are available in the form of hydrolysable tannins called as ellagitannins. The aim of the present study is to develop and validate a simple, fast, specific and precise HPTLC method for the estimation of ellagic acid in fresh and processed fruit products. Aluminium plates precoated with silica gel 60 GF_{254} was used as stationary phase and a mixture of Toluene: Ethyl Acetate: Formic acid in the ratio of 5: 5: 2.5 (v/v) as mobile phase. Quantitation was carried out by scanning the developed spots using a densitometer in absorbance mode at 254 nm. The Rf value of Ellagic acid was 0.35. The result of analysis has been validated statistically and by recovery studies. Linearity was observed in the concentration range of 400 – 1200 ng/spot. HPTLC method for the estimation of ellagic acid was developed and validated. Thus it may be used for the estimation of ellagic acid in fresh and processed fruits.
KEY WORDS: Ellagic acid, HPTLC, Fresh fruits, Processed fruit products.

INTRODUCTION

It is well established that a diet high in fruits and vegetables is associated with a reduced risk of oxidative stress mediated diseases such as cancer, cardiovascular and neurodegenerative diseases. The health beneficial effects of fruits and vegetables are attributed to their high levels of a wide variety of phytochemicals, of which phenolics constitute the greatest proportion. Among them, Ellagic acid, a dimeric compound of gallic acid is found in numerous fruits and vegetables including raspberries, strawberries, cranberries, walnuts, pecans, pomegranates and other plant foods in the form of hydrolysable tannins called ellagitannins. Ellagitannins are esters of glucose with hexahydroxydiphenic acid; when hydrolyzed, they yield Ellagic acid, the dilactone of hexahydroxydiphenic acid. Ellagic acid is found to have antiviral, antimutagenic and antioxidant properties. The anti proliferative and antioxidant properties of ellagic acid have spurred preliminary research into the potential health benefits of ellagic acid consumption.

Detailed review of literature for various analytical methods for the detection of ellagic acid revealed several methods based on different techniques Viz LC-ESI-MS[1, 2] for the estimation of phenolic compounds in strawberry fruits and identification of ellagic acid conjugates and other polyphenolics in muscadine grapes. An LCMS[3] method was also reported for the analysis of ellagitannins and quercetin in raspberry fruits. HPLC[4, 5] methods were also carried out to estimate the total content of phenolics and ellagic acid in fresh and processed fruits. A survey[6] was also reported for the content of ellagic acid in selected foods consumed in Finland. However there was no HPTLC method reported for the determination of ellagic acid in fresh and processed fruits. The purpose of the present study was to evaluate ellagic acid in fresh and processed fruits available in Indian market by an accurate and sensitive HPTLC method. The developed method was validated as per ICH guidelines[7].

EXPERIMENTAL

Solvents and chemicals

Ellagic acid was procured from sigma Aldrich limited, India. Food products were procured commercially. Chromatographic grade solvents like methanol, chloroform, acetone and formic acid were obtained from Qualigens chemicals, Mumbai, India.

Standard and sample solutions

Ellagic acid (10 mg) was accurately weighed into a 100 ml volumetric flask, dissolved in methanol and diluted up to the volume with same solvent. The stock solution was stored in light resistant containers. This stock solution was further used to furnish working standards.

Ellagic acid from four different food products (pomegranate fruit, strawberry fruit, strawberry jam, raspberry jam) were extracted by hydrolysis[5] of 10 g of the samples with 30 ml of methanol, refluxed for 1 hour and filtered using a Whatmann filter paper No 42. 10 ml of distilled water was added to the filtrate and evaporated to a volume of 10 ml. 0.25 ml of 0.1N hydrochloric acid was added and the volume was made up to 25 ml with distilled water. Ellagic acid from this solution was separated using solid phase extraction technique. Phenomenex Strata C_{18} columns with 1 ml capacity were used for SPE analysis. The catridges were conditioned using 1ml of methanol and water, 1 ml of the refluxed sample was loaded and the samples were extracted with 2 ml of methanol and used for analysis.

Chromatography

Chromatography was performed on an aluminum backed silica gel 60 GF_{254} TLC plates pre-washed with methanol. (0.2 mm Thickness, E Merck, Darmstadt, Germany).

Standard solutions of ellagic acid were prepared by transferring the stock solutions in different 10 ml volumetric flasks and diluted to the volume with methanol such that the concentrations are 0.4-1.200 µg/µl. The standards and four different sample solutions were applied to the TLC plates as 8.0 mm bands with 9.0 mm space between two bands using a

Camag Linnomat IV sample applicator. The plates were developed with a mobile phase of Toluene: Ethyl acetate: formic acid [5:5:2.5 % v/v] in a TLC twin trough chamber previously saturated with the solvent for 30 minutes. After development the plates were dried at 60ºC for 5 minutes and the quantification of the standards and samples were performed by means of a Camag TLC scanner III controlled by WinCATS 1.4.3 version software at 254 nm. The amount of ellagic acid in the sample solutions were computed from the calibration plot (Figure 1-5).

Method validation

Linearity

Standard solutions of 400 – 1200 ng/spot of ellagic acid were analyzed to check the linearity of response.

Precision

Repeatability of the sample application and measurement of peak area were carried out by spotting six replicates of three different concentration of ellagic acid (400, 800 and 1200 ng/spot) and was expressed in terms of percent relative standard deviation (% RSD).

Accuracy

Accuracy of the method was determined by recovery experiments. The recovery of the method was determined at single level by adding a known quantity of ellagic acid to the food products of pre analyzed samples and the mixtures were reanalyzed.

Ruggedness and robustness

The ruggedness of the proposed method was determined by carrying out the experiment with different analyst, calculation of % recovery of ellagic acid. Robustness of the method was determined by making small changes in the chromatographic conditions like variation in the solvent front.

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the developed HPTLC method were detected based on the signal to noise ratios. For LOD the signal to noise ratio was 3:1 and for LOQ as 10:1.

RESULTS AND DISCUSSION

The selected mobile phase of Toluene: Ethyl acetate: Formic acid [5:5:2.5 % v/v] resolves Ellagic acid efficiently and is shown in fig. I. The R_f value of ellagic acid was found to be 0.35. Ellagic acid solution gave an absorbance maximum at 254 nm and was selected for detection. The method was used to determine ellagic acid content in two fresh fruits and two processed fruit products. The results were tabulated in Table-1.

System suitability

System suitability tests are an integral part of a chromatographic analysis and should be used to verify that the resolution and reproducibility of the chromatographic systems are adequate for analysis. To ascertain the effectiveness of the method developed, system suitability tests were performed on a freshly prepared standard stock solution of ellagic acid.

Linearity

A calibration plot of peak area against concentration of ellagic acid was linear in the concentration range of 400 to 1200 ng. The calibration lines were represented by the linear regression equation Y = 1298+4.942 X where Y is the peak area and X is concentration. The correlation coefficient r^2 was found to be 0.998.

Accuracy

The accuracy and precision of the method were studied by performing experiments by standard addition methods. Accuracy of the method was determined by recovery experiments. The recovery of the method was determined at single level by adding a known quantity of ellagic acid to the food products of pre analyzed samples and the mixtures were analyzed according to the proposed method. The average recovery obtained from each sample was between 98.37 and 102.83% and is shown in Table-1. From the data obtained, added recovery of standard drug was found to be accurate.

Limit of quantification and detection

The Limit of Quantification (LOQ) and limit of Detection(LOD) were calculated by use of the equations LOD = 3 x N/B and LOQ = 10 x N/B where N is the standard deviation of the peak area of the drug, taken as a measure of the noise and B is the slope of the corresponding calibration curve. The limit of quantification and the limit of detection for ellagic acid were found to be 3.16 ng and 1.04 ng (Table-2).

Precision

The relative standard deviations for repeatability of sample application and repeatability of peak area were calculated. The precision measurements of peak area and instrumental variations for different concentration levels were found to be < 1.5% of % RSD which indicates the excellence of the method precision (Table-3).

Ruggedness and robustness

Ruggedness is the measure of the reproducibility of a test result under normal, expected operating conditions from instrument to instrument and from analyst to analyst (Table-4). Robustness of the method was determined by making slight changes in the chromatographic conditions. No marked changes in the chromatograms demonstrated that the HPTLC method developed are rugged and robust (Table-5).

CONCLUSION

The HPTLC method proposed for the determination of Ellagic acid in two different fresh fruits and two different processed fruit products were accurate, precise, rapid, selective and sensitive and therefore can be conveniently adopted for the routine analysis of ellagic acid in food products.

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Table: 1. Analysis of ellagic acid in fresh and processed food products

S. NO	SAMPLE	AMOUNT OF ELLAGIC ACID PRESENT [mg ±RSD*]/ 10 GM	RECOVERY [%±RSD*]
1	Strawberry fruit	50.06 ± 0.1364	98.46 ± 01981
2	Pomegranate fruit	19.81 ± 0.2355	102.83 ± 0.2576
3	Strawberry Jam	95.88 ± 0.1469	102.45 ± 0.1240
4	Raspberry Jam	86.87 ± 0.2046	98.37 ± 0.1552

*RSD of three determinations

Table: 2. System suitability studies

S. NO	PARAMETERS	ELLAGIC ACID
1	Linearity Range	400 – 1200 ng/spot
2	Regression equation Y = mX+C	Y = 4.942X + 1298
3	Correlation coefficient	0.998
4	LOD (ng)	1.04
5	LOQ (ng)	3.16

Table: 3. Precision studies for Ellagic acid by HPTLC

S. No	Ellagic acid		
	400 ng/spot	800 ng/spot	1200 ng/spot
1	3224.2	5320.8	7153.9
2	3256.8	5368.1	7197.8
3	3329.5	5296.5	7105.3
4	3197.6	5423.7	7065.2
5	3260.8	5413.5	7134.9
6	3284.1	5387.6	7225.7
Mean	3258.83	5368.36	7147.13
SD	46.02	50.78	58.98
% RSD	1.4121	0.9460	0.8253

Table: 4. Results from ruggedness studies*

Analysts	Amount recovered [%]			
	Strawberry fruit	Pomegranate fruit	Strawberry jam	Raspberry jam
Analyst I	98.46	102.83	102.45	98.37
Analyst II	99.12	101.65	100.86	99.41

* All the values are % recovery in food products

Table: 5. Results from robustness studies

Development distance [mm]	Amount recovered [mg]			
	Strawberry fruit	Pomegranate fruit	Strawberry Jam	Raspberry Jam
75.0	98.85	101.32	101.45	99.46
80.0	100.26	102.98	102.75	99.03

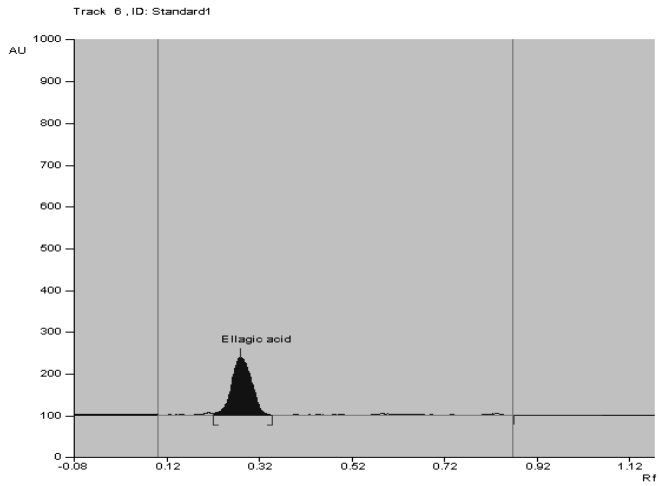

Fig: 1. Typical HPTLC Chromatogram of Standard Ellagic acid

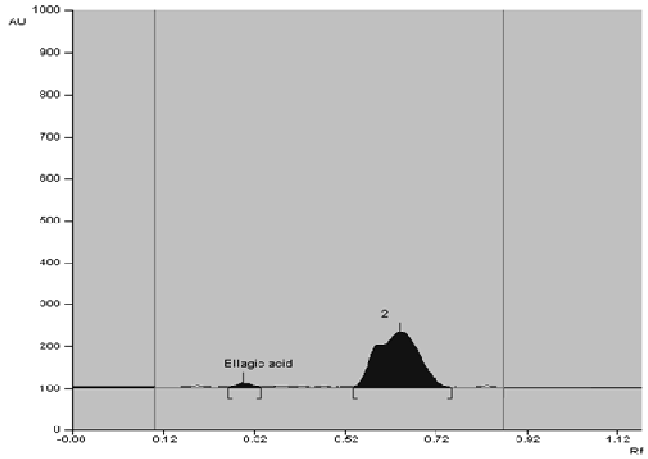

Fig: 2. Typical HPTLC Chromatogram of Ellagic acid in strawberry fruit

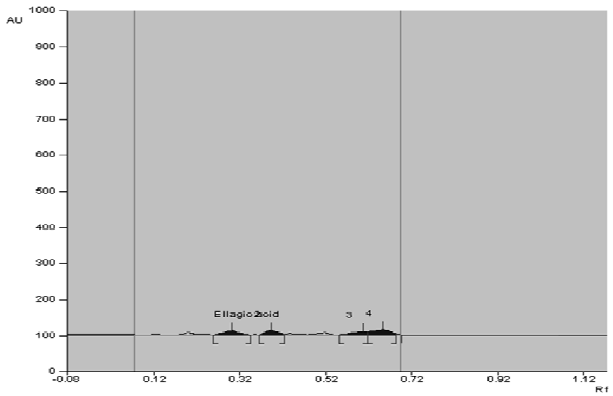

Fig: 3. Typical HPTLC Chromatogram of Ellagic acid in Pomegranate fruit

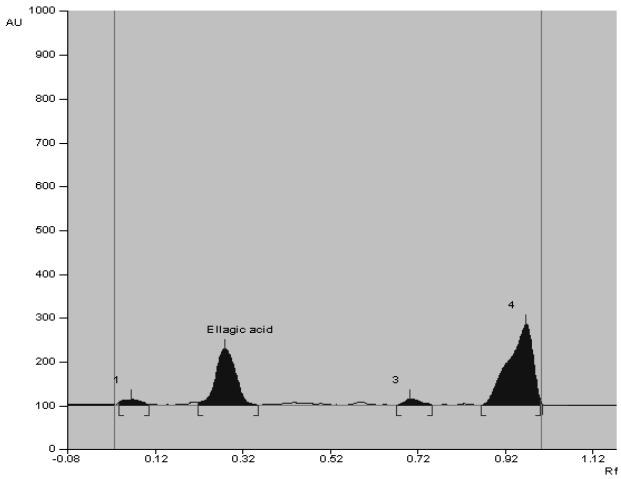

Fig: 4. Typical HPTLC Chromatogram of ellagic acid in strawberry jam

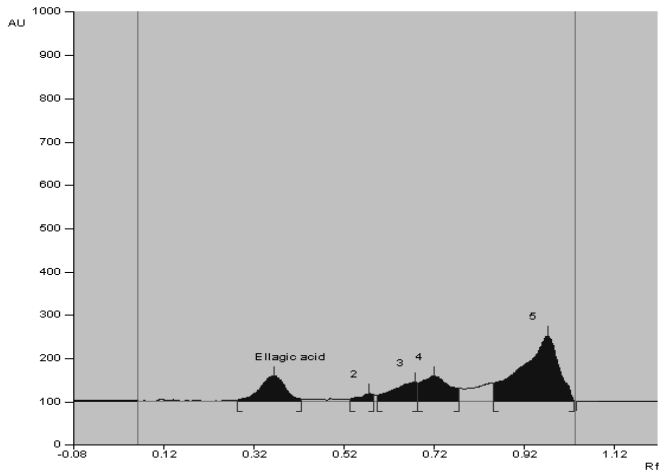

Fig: 5. Typical HPTLC Chromatogram of Ellagic acid in Raspberry jam

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