Original Article

Simultaneous assessment and validation of reverse phase-high performance liquid chromatography method for quercetin, eugenol, myristicin, and safrole from nutmeg, fruit and mace

Abstract

Background: Nutmeg is the imperative spices having pharmacological importance. **Objectives:** The objective of this work was to standardize Nutmeg extract by RP-high performance liquid chromatography (HPLC) analysis. **Settings and Design:** An RP-HPLC method was developed for simultaneous quantification of quercetin (QUE), eugenol (EUG), myristicin (MYRS), and safrole (SAFR) from nutmeg fruit and mace extracts. **Materials and Methods:** RP-HPLC method was performed with Waters 2695 Alliance system using a 2996 photodiode array detector (PDA). QUE, EUG, MYRS, and SAFR were separated on a reverse-phase 250 × 4.6 mm, 5-μm, Zorbax SB C18 column (Agilent). The mobile phase was prepared from 0.1% orthophosphoric acid in water of pH 2.5 (solvent-A) and acetonitrile (solvent-B). The gradient program was selected for separation. The PDA was set at 220 nm, which shows maximum response for all peaks. **Statistical Analysis:** Percent relative standard deviation (% RSD) and correlation coefficient (r^2) were calculated by standard formulas. **Results:** QUE, EUG, MYRS, and SAFR were satisfactorily resolved with retention time about 3, 7, 19 and 21 min. respectively. The method was validated and results obtained showed accepted values for correlation of coefficient and % RSD. **Conclusions:** The method was accurate and specific for analysis of nutmeg extract.

Key words:
Eugenol, myristicin, myristica fragrans, quercetin, RP-HPLC, safrole standardization

Introduction

Myristica fragrans Houtt. (Myristicaceae) is an aromatic evergreen tree cultivated in South Africa, India, and other tropical countries. Nutmeg is the kernel of the seed of the plant *M. fragrans* and mace is the dried Aril that surrounds the seed within the nutmeg fruit. Both nutmeg (seed) and mace are used as medicine and spices with similar tastes. The former is sweeter whereas the latter is known to release more delicate flavors. It is used in numerous recipes, including a lot of desserts (e.g., fruit cakes, muffins, pies) but in main courses as well (e.g., potato dishes, sauces). Nutmeg is also added to beverages (e.g., tea, mulled wine) and it is an ingredient of some curry powders. After nutmeg, which refers to the dried kernels of this plant, was imported into Europe at the 12th century; it has long been used indigenously as a spice in many Western foods. Nutmeg is also prescribed for medicinal purposes in Asia to treat many diseases, such as rheumatism, muscle spasm, decreased appetite, and diarrhea.[1,2] *M. fragrans* Houtt. (Myristicaceae), known as pala in Indonesia, luk jan in Thailand, nikuzuku in Japan, and commonly nutmeg or mace, has been used traditionally for spice and medicinal purposes for carminative, hypo-lipidemic, anti-thrombotic, anti-platelet aggregating, anti-fungal, aphrodisiac, anxiogenic, anti-ulcerogenic, antitumor, anti-inflammatory activities, etc.,[3-6] Spices are considered as sexual invigorators in the Unani System of Medicine.[7]

The major constituents of nutmeg are eugenol (EUG), myristicin (MYRS), safrole (SAFR), trimyristicin, etc., Apart from

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that quercetin (QUE), β-pinene, elemicin, and methyl-eugenol are also present in different part of the nutmeg plant.[8]

EUG, used as an analgesic agent in dentistry, is reported to have antiseptic and anodyne activities, as well as myorelaxant and anticonvulsant actions. There is strong evidence that EUG inhibits the synthesis of prostaglandins and that it reduces vasoconstrictor responses to norepinephrine, histamine, and stimulation of peri-arterial sympathetic nerves, responses that are associated with pain,[9] and also exhibits antidepressant-like activity.[10]

QUE is one of the most important flavonoid and occurs in food as aglycone (attached to a sugar molecule). QUE has many health promoting effects, including anti-inflammatory and anti-allergic effects, as well as improvement of cardiovascular health and reducing risk for cancer. All these activities are caused by the strong antioxidant action of QUE. It will help to combat free radicals, which can damage cells. As many other flavonoids, QUE prevents the oxidation of low-density lipoprotein cholesterol[11] and has anti-inflammatory activity.[12]

In contrast with that, SAFR a naturally occurring plant constituent, which is hepatotoxic and shows weak hepatocarcinogenic activity, and when fed to rats and mice induces high incidences of liver tumors. SAFR is no longer used as a food additive in the United States and many other countries, but because of its occurrence in certain spices and other plant derivatives, this compound is ingested in small amounts by many humans.[13]

Not only SAFR but also MYRS toxicity is considerable. MYRS is a 4 methoxy derivative of safrole which induces the enzyme glutathione-*S*-transferase[14] furthermore, it has weak monoamine oxidase properties that may be responsible for cardiovascular symptoms. It has been shown to have hypotensive, sedative, anti-depressant, anesthetic, hallucinogenic, and serotonergic properties, and in large doses it generally causes hyper-excitability followed by central nervous system depression.[15]

Nutmeg contains both types of chemical constituents, pharmacologically beneficial such as EUG, and QUE, and pharmacologically toxic such as MYRS and SAFR, so that safety evaluation is caught in a frustrating circle for daily use of nutmeg as spices.

According to the literature, very few analytical methods are available for analysis of MYRS and SAFR from nutmeg such as high-performance liquid chromatography (HPLC) determination of SAFR and MYRS in nutmeg and mace,[16] and high-performance thin-layer chromatography (HPTLC) analysis of SAFR and MYRS in seed powder of nutmeg,[17] and no method available for simultaneous estimation of QUE, EUG, SAFR, and MYRS, which will prove fruitful to estimate efficacy and safety by the same HPLC method.

Therefore, attempt has been made toward standardization of nutmeg by developing a simple, accurate, sophisticated RP-HPLC method for analysis of these chemical constituents by a single method, which can be useful for routine analysis.

Materials and Methods

Plant material

The fruit and mace of nutmeg were purchased from the local market of Pune region of Maharashtra. Crude material was authenticated and submitted to Tulip Lab Pvt. Ltd., Pune, India, with voucher specimen no. TLPLMFF-01 and TLPLMFM-02 for nutmeg fruit and mace, respectively. It was dried and powdered in a mill. The powdered crude material of fruit and mace of nutmeg was passed through sieve no. 85, weighed, and then used for extraction separately.

Preparation of extracts

The powdered fruit and mace of nutmeg were extracted with various solvents in increasing order of polarity such as petroleum ether, ethyl acetate, chloroform, methanol, and water: Methanol (1:1) for 36 h at temperature near to boiling point of the respective solvents [Table 1] by using soxhlet apparatus separately. These extracts were then concentrated to dryness by removing the solvent in the rotary evaporator under reduced pressure. The respective instrumental conditions such as temperature and pressure were set at the time of concentration of extracts in a rotary evaporator [Table 2].

Determination of plant extract yield

The % yield of extracts obtained from nutmeg fruit and mace was calculated as dry basis by using Loss on Drying (LD) [Table 3] of individual extracts after triplicate extraction using the following equation:

$$\% \text{ Yield} = W1 \times (100\text{-LD}) \times 100/W2$$

Table 1: Temperature and polarity index of extraction solvents

Solvent	Polarity index	Temperature (°C)
Petroleum ether	0.0	35
Ethyl acetate	4.4	77
Chloroform	4.1	61
Methanol	5.1	65
Water: Methanol (1:1)	9.0	80

Table 2: Rotary evaporator instrumental conditions for extraction solvents

Solvent	Pressure (mBar)	Temperature (°C)
Petroleum ether	360	60
Ethyl acetate	240	60
Chloroform	474	60
Methanol	377	60
Water: Methanol (1:1)	72	60

Where W1 is the weight of the extract after the solvent evaporation, W2 is the weight of powdered nutmeg fruit and mace taken, respectively, and LD is Loss on Drying of extract. The result is expressed in mean±SD.

Chemicals

HPLC-grade solvents such as petroleum ether, chloroform, acetonitrile, methanol and water were obtained from Merck Ltd. Bangalore India. Standards of QUE (potency 96% w/w, product no. Q4951), EUG (potency 99% w/w, product no. E51791), SAFR (potency 98.5% w/w, product no. S9652), and MYRS (potency 98% w/w product no. 09237) were purchased from Sigma, Bangalore, India.

Preparation of mixture of standard solution

Stock solutions of QUE (500 μg/ml), EUG (180 μg/ml), SAFR (2700 μg/ml), and MYRS (1500 μg/ml) were prepared in methanol separately. Mixed standard solution was prepared by diluting 1 ml of each standard solution upto 10 ml with methanol. The solution was filtered through a 0.45-μm syringe filter and the resulting solution was used as standard solution.

Preparation of sample for analysis

A 500-mg weight each extract of fruit and mace of nutmeg was accurately weighed and dissolved in 50 ml of methanol separately. These resulting solutions were used for analysis. Analysis was done in triplicate.

Chromatographic conditions for HPLC

HPLC was performed using a Waters 2695 Alliance system with a 2996 photodiode array detector (PDA). QUE, EUG, SAFR, and MYRS were separated on a reverse-phase 250 × 4.6 mm, 5-μm, Zorbax SB C18 column (Agilent, Mumbai, India. The mobile phase was prepared from 0.1% orthophosphoric acid in water of pH 2.5 (solvent-A) and acetonitrile (solvent-B). The mobile phase was degassed and filtered through a 0.45-μm filter before use. The gradient program used is given in Table 4. The mobile phase flow rate was 1 ml/min. Before the first injection, the column was saturated for 30 min with the initial mobile phase. Temperature was maintained at 35°C. Injection volume was kept maintained 10 μl. The PDA was set by optimizing wavelength to give best response for all four peaks at 220 nm to acquire the chromatogram. QUE, EUG, SAFR, and MYRS were identified by comparing the retention time and spectra obtained from the sample and standard solutions. The present work was performed in an air-conditioned room maintained at 25°C. The methanolic extract of the fruit of nutmeg was used for validation.

Preparation of calibration graph

From the mixture of the working standard solution seven different concentrations were prepared by diluting 1, 1.3, 1.6, 2, 2.3, 2.6, and 3 ml up to 20 ml with methanol, and these were injected into the system. The calibration plot of each standard was constructed by plotting concentrations against peak area for the respective standards.

Validation of HPLC method

The proposed HPLC method was validated in terms of specificity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), standard solution stability, sample solution stability, and robustness as per the International Conference on Harmonization (ICH) guidelines.[18]

Specificity

The specificity of the method was studied by assessment of peak purity of QUE, EUG, SAFR, and MYRS using the Waters empower software and diode array detector [Figure 1] and represented in terms of purity angle, purity threshold, and purity flag [Table 5].

Table 3: LD and % yield of various extracts

Part used	Type of extract	LD (%) (mean SD)	% yield (mean±SD)
Nutmeg fruit	Petroleum ether	8.4±0.01	5.5±0.03
	Ethyl acetate	10.9±0.02	13.45±0.01
	Chloroform	11.2±0.01	12.20±0.02
	Methanol	11.8±0.03	24.53±0.01
	Water: Methanol (1:1)	12.5±0.01	20.12±0.05
Nutmeg, mace	Petroleum ether	7.9±0.01	4.7±0.02
	Ethyl acetate	10.1±0.02	11.24±0.01
	Chloroform	11.9±0.01	10.35±0.03
	Methanol	12.1±0.04	17.54±0.02
	Water: Methanol (1:1)	12.7±0.02	15.80±0.01

LD – Loss on drying; SD – Standard deviation

Table 4: Gradient program for mobile phase

Time	Flow (ml/min)	Solvent-A%	Solvent-B%
0	1.0	50	50
8	1.0	50	50
9	1.0	60	40
35	1.0	35	65
40	1.0	35	65
41	1.0	05	95
45	1.0	05	95
46	1.0	50	50
50	1.0	50	50

Table 5: Specificity parameters

Standard	Purity angle	Purity threshold	Purity flag
Quercetin	0.208	0.265	No flag found*
Eugenol	0.506	1.230	No flag found*
Myristicin	0.104	0.260	No flag found*
Safrole	0.197	0.266	No flag found*

No flag found* means no interference in standard peak

Precision

Precision was studied in terms of system precision, method precision, and intermediate precision.

System precision

System precision was carried out by six replicate injections from the same vial of standard and was expressed in terms of percent relative standard deviation (% RSD) tailing, plate count, and resolution [Table 6].

Method precision

Method precision was done at two different concentrations, one at working concentration and other at LOQ. It was done by analyzing six different sample of extract at both concentrations and was expressed in terms of % RSD, tailing, and plate count [Table 7].

Intermediate precision

Intermediate precision was performed on different systems, one the Waters 2695 Alliance system with a 2996 PDA and the other a 2489 ultraviolet (UV) detector by different analysts by analyzing six different sample of extract and was expressed in terms of % RSD [Table 8].

Recovery studies

The accuracy of the method was determined from recovery studies by adding a known amount of each standard at the 80%, 100%, and 120% level to the pre-analyzed sample followed by replicate quantitative analyses by the proposed method [Table 9].

Table 6: System precision parameters

Standard	% RSD	Tailing	Plate count	Resolution
Quercetin	1.10	0.90	4665	–
Eugenol	1.21	1.10	12295	17.60
Myristicin	1.29	1.06	11623	24.94
Safrole	1.38	1.05	16019	3.0

RSD – Relative standard deviation

Table 7: Method precision parameters

Concentration	Analyte	% RSD	Tailing	Plate count
LOQ	Quercetin	1.20	0.95	4325
	Eugenol	1.11	1.01	11,995
	Myristicin	1.25	0.97	11,221
	Safrole	1.40	1.11	15,598
Working concentration	Quercetin	1.97	0.91	4132
	Eugenol	1.05	1.05	12,029
	Myristicin	1.29	0.91	11,954
	Safrole	1.35	1.01	16,024

RSD – Relative standard deviation; LOQ – Limit of quantification

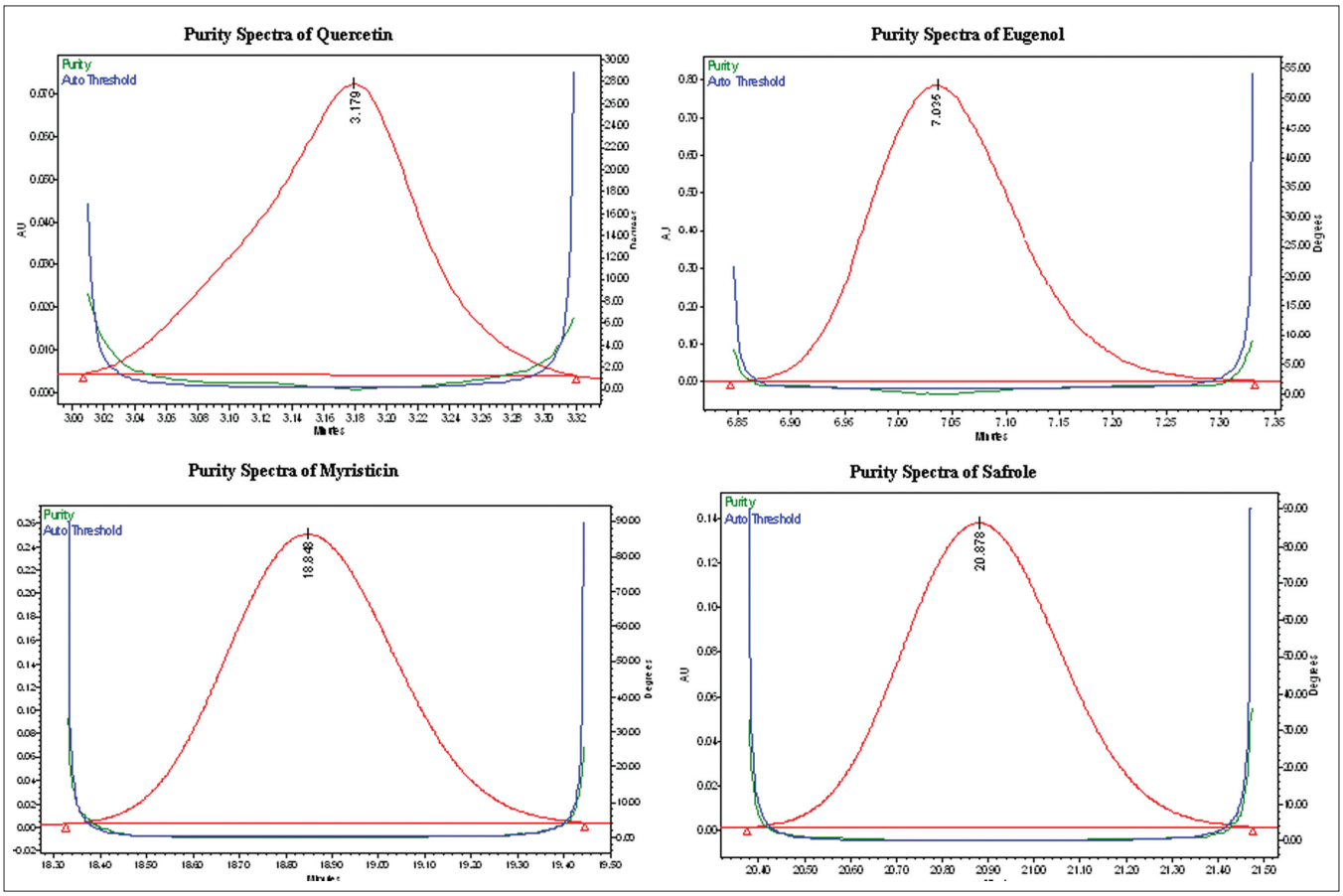

Figure 1: Purity spectra for specificity

Analytical solution stability

The standard solution and sample solution were prepared as per the proposed method and subjected to stability study at room temperature for 48 h. The sample solution was analyzed at initial and at different time intervals of 4 h up to 48 h. Change in the response of QUE, EUG, SAFR, and MYRS in the sample solution with respect to time was calculated as absolute percent difference against initial response [Tables 10 and 11].

Robustness

Robustness of the method was determined by slight deviation in the method parameters. The parameters selected were deviation in column chemistry, wavelength, column temperature, flow rate, and mobile phase gradient. The retention time of QUE, EUG, SAFR, and MYRS, respectively, was determined and % RSD using system suitability parameters was observed [Table 12].

LOD and LOQ

LOD and LOQ were determined based on the standard deviation of the response and the slope of the calibration curve at low concentration levels according to ICH guidelines. It was determined by plotting a calibration graph of the respective standard solution at low concentration and calculated by using the following equations:

$$LOD = 3.3\sigma/S$$

$$LOQ = 10\sigma/S;$$

σ = standard deviation of response;
S = slope of calibration curve

Table 8: Intermediate precision

Analyst	QUE (% w/w)	EUG (% w/w)	SAFR (% w/w)	MYRS (% w/w)
Analyst-1	0.532	0.185	2.77	1.51
	0.541	0.182	2.67	1.54
	0.534	0.184	2.73	1.55
	0.549	0.184	2.69	1.49
	0.537	0.181	2.76	1.57
	0.554	0.187	2.64	1.54
Analyst-2	0.557	0.181	2.74	1.58
	0.539	0.179	2.65	1.52
	0.537	0.191	2.72	1.51
	0.536	0.184	2.69	1.52
	0.548	0.181	2.75	1.56
	0.532	0.185	2.77	1.51
Average	0.54	0.183	2.7	1.5
% RSD	1.55	1.82	1.65	1.82

EUG – Eugenol; MYRS – Myristicin; QUE – Quercetin; SAFR – Safrole; RSD – Relative standard deviation

Table 9: Recovery studies

Analyte	Recovery level (%)	Amount added (mg)	Amount recovered (mg)	% Recovery	% Average recovery
Quercetin	80-1	0.40	0.399	99.75	99.71
	100-2	0.51	0.506	99.22	
	120-3	0.60	0.601	100.17	
Eugenol	80-1	0.15	0.149	99.33	99.56
	100-2	0.18	0.178	98.89	
	120-3	0.22	0.221	100.45	
Safrole	80-1	2.21	2.19	99.10	98.48
	100-2	2.77	2.72	98.19	
	120-3	3.24	3.18	98.15	
Myristicin	80-1	1.22	1.21	99.18	98.96
	100-2	1.51	1.50	99.34	
	120-3	1.84	1.81	98.37	

Table 10: Standard solution stability

Time in hours	QUE		EUG		SAFR		MYRS	
	Area	APDI	Area	APDI	Area	APDI	Area	APDI
0	507024	–	7553120	–	3445104	–	6345914	–
4	506414	0.12	7552154	0.01	3455104	0.29	6340101	0.09
8	506414	0.42	7571245	0.23	3454214	0.26	6337541	0.13
12	504875	0.23	7527652	0.33	3422541	0.65	6350214	0.06
16	508214	0.44	7518547	0.45	3419547	0.74	6298541	0.74
20	504781	0.62	7498745	0.72	3407541	1.09	6274514	1.12
24	503863	1.1	7454772	1.30	3397541	1.38	6241542	1.64

APDI – Absolute percent difference from initial; EUG – Eugenol; MYRS – Myristicin; QUE – Quercetin; SAFR – Safrole

Table 11: Sample solution stability

Time in hours	QUE		EUG		SAFR		MYRS	
	Area	APDI	Area	APDI	Area	APDI	Area	APDI
0	153375	–	321147	–	401699	–	1993298	–
4	153197	0.12	320874	0.03	402367	0.02	1988254	0.02
8	154754	0.23	319861	0.04	402179	0.14	1985471	0.14
12	152612	0.77	318745	0.36	400828	0.09	1990937	0.09
16	151147	0.61	316191	0.71	400021	0.24	1987541	0.24
20	151279	1.57	314769	1.50	397847	0.44	1979814	0.44
24	153375	1.48	321147	1.94	401699	0.98	1993298	0.98

APDI – Absolute percent difference from initial; EUG – Eugenol; MYRS – Myristicin; QUE – Quercetin; SAFR – Safrole

Table 12: Robustness of the method

Parameters	% RSD			
	QUE	EUG	SAFR	MYRS
Wavelength	0.03	0.05	0.06	0.04
Column temperature	0.21	0.15	0.27	0.31
Flow rate	0.61	0.41	0.55	0.52
Mobile phase gradient	0.34	0.21	0.12	0.47
Mobile phase pH	0.41	0.15	0.22	0.52
Column chemistry	0.59	0.89	0.19	1.23

EUG – Eugenol; MYRS – Myristicin; QUE – Quercetin; RSD – Relative standard deviation; SAFR – Safrole

Analysis of extract

Petroleum ether, chloroform, and methanol extracts of both fruit and mace were analyzed separately to determine the contents of QUE, EUG, SAFR, and MYRS as per the method described under chromatographic conditions by HPLC. All analysis were repeated three times and results were expressed in mean±SD.

Results and Discussion

Estimation of % yield of extracts

The percent yield of each extract of fruit and mace of nutmeg was determined and is given in Table 3. The % yield of fruit was 5.5±0.03, 13.45±0.01, 12.20±0.02, 24.53±0.01, and 20.12±0.05, and that of mace was 4.7±0.02, 11.24±0.01, 10.35±0.03, 17.54±0.02, and 15.80±0.01 for petroleum ether, ethyl acetate, chloroform, methanol and water: Methanol (1:1) respectively.

The methanolic extract of nutmeg fruit showed highest % yield among all extracts.

Chromatographic study

The composition of the mobile phase in the HPLC method was optimized by testing different solvent compositions of varying polarity, column chemistry, column temperature, and pH of mobile phase, and the best results were obtained by using the present method, which produces highly symmetrical peaks showing good resolution between each standard and other peaks [Figure 2]. The scanning wavelength selected was 220 nm to provide comparable results and at this wavelength all standards showed optimum response [Figures 3-5]. QUE, EUG, MYRS, and SAFR were satisfactorily resolved with retention time about 3, 7, 19, and 21 min, respectively.

The calibration graph was linear in the working range of 50-150 μg/ml, with acceptable correlation coefficients 0.9996, 0.9998, 0.9995, and 0.9995 for QUE (25-75 μg/ml), EUG (9-27 μg/ml), SAFR (135-405 μg/ml), and MYRS (75-225 μg/ml), respectively [Table 13]. The graph for each standard is given in Figure 6.

The values of system precision, method precision, and intermediate precision are given against sample application and scanning of peak area and are expressed in terms of % RSD.

For system precision %RSD values were found to be 1.10 %, 1.21%, 1.29%, and 1.38% for QUE, EUG, MYRS, and SAFR [Table 6].

Method precision was done at two concentration level, at LOQ level and at working concentration. % RSD value was found to be 1.20%, 1.11%, 1.25%, and 1.40% at LOQ level and 1.97%, 1.05%, 1.29%, and 1.35% at working concentration for QUE, EUG, MYRS, and SAFR, respectively [Table 7].

For intermediate precision % RSD values between the two analysts were found to be 1.55%, 1.82%, 1.65%, and 1.82% for QUE, EUG, SAFR, and MYRS, respectively [Table 8].

For the values of system precision, method precision, and intermediate precision, the % RSD values showed that the proposed method provides an acceptable level of system precision, method precision, and intermediate precision.

The peak purity of for each analyte was assessed by comparing their respective spectra at peak start, peak apex, and peak end positions of the spot from standard and extracts [Figures 1 and 3]. The purity angle and purity threshold values are given in table [Table 5].

Table 13: Limit of detection, limit of quantification, and linearity study

Standard	LOD (µg/ml)	LOQ (µg/ml)	Linearity (correlation coefficient=r^2)
Quercetin	6.06	18.39	0.9996
Eugenol	2.15	6.51	0.9998
Myristicin	1.81	5.54	0.9995
Safrole	1.13	3.72	0.9995

LOD – Limit of detection; LOQ – Limit of quantification

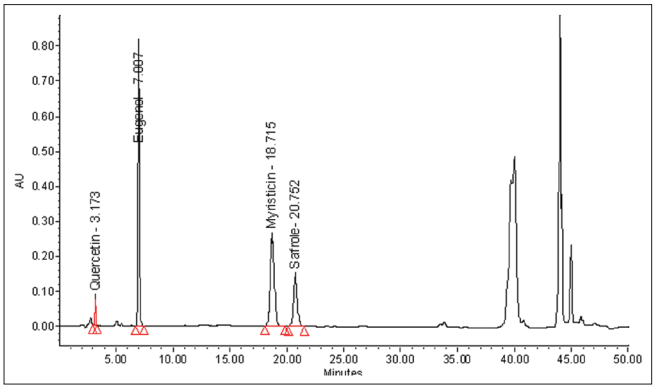

Figure 2: Standard chromatogram of mixed standard

The given method was optimized by doing robustness. The peak area for each analyte was calculated for each parameter and % RSD was found to be less than 2%. The values of % RSD as shown in Table 12 indicate better robustness of the method.

The proposed method was used for estimation of QUE, EUG, SAFR, and MYRS from extract after spiking with 80%, 100%, and 120% of additional standards, respectively, to a pre-analyzed sample. The recovery percent for QUE were found to be 99.71%, for EUG 99.56%, for SAFR 98.48%, and for MYRS 98.96% [Table 9].

LOD and LOQ were determined based on the standard deviation of the response and the slope of the calibration curve at low concentration levels of each analyte separately. The LOD and LOQ values for each analyte are given in Table 13. The values for LOD and LOQ were found to be 6.06 µg/ml and 18.39 µg/ml, for QUE, 2.15 µg/ml, and 6.51 µg/ml, for EUG, 1.81 µg/ml and 5.54 µg/ml for SAFR, and 1.13 µg/ml and 3.72 µg/ml for MYRS, respectively.

Analytical solution stability was done on standard solution

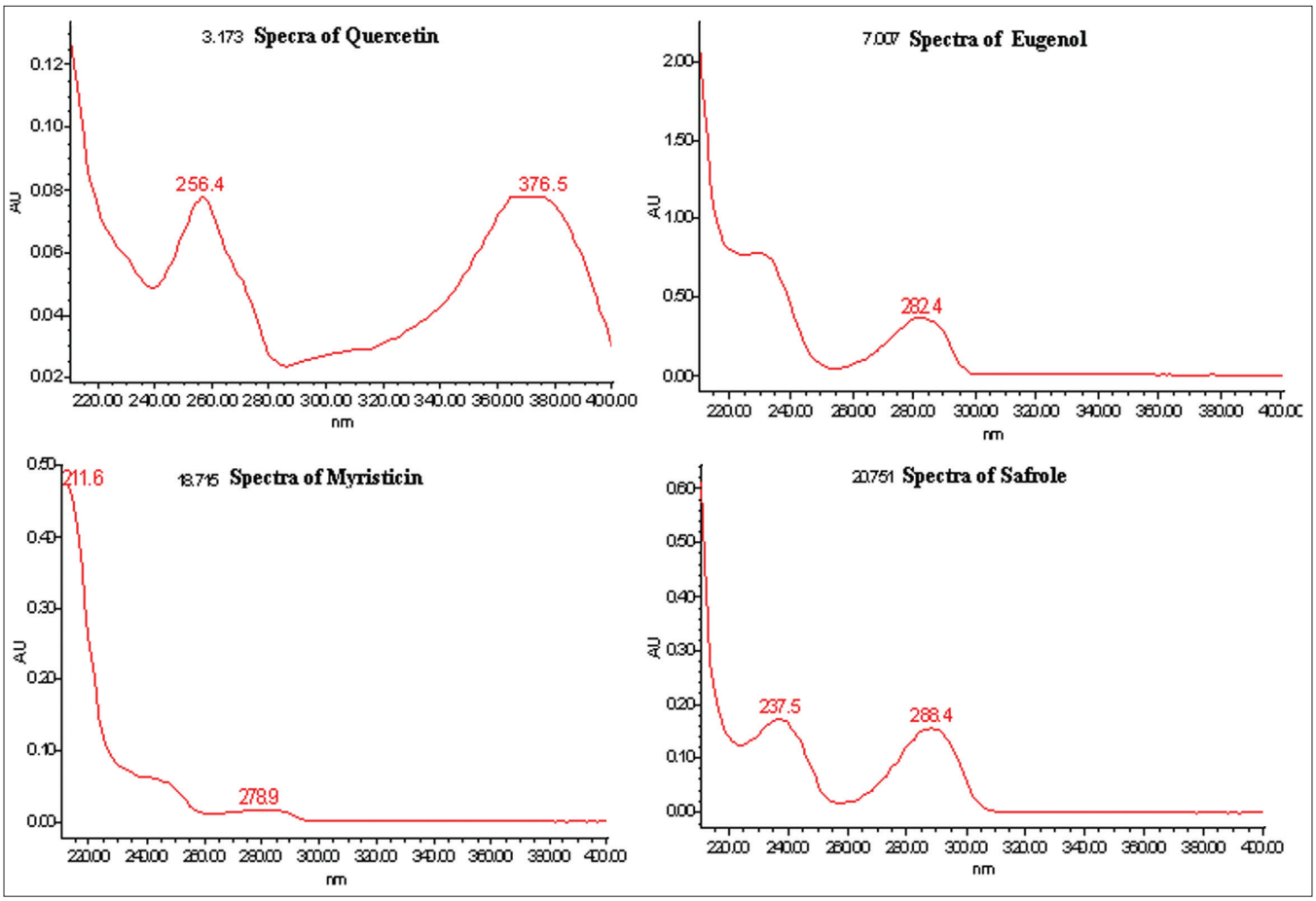

Figure 3: Spectra of standard

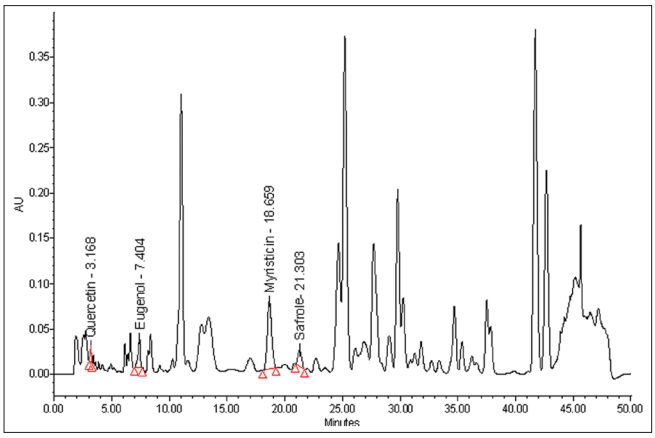

Figure 4: Chromatogram of extract of mace

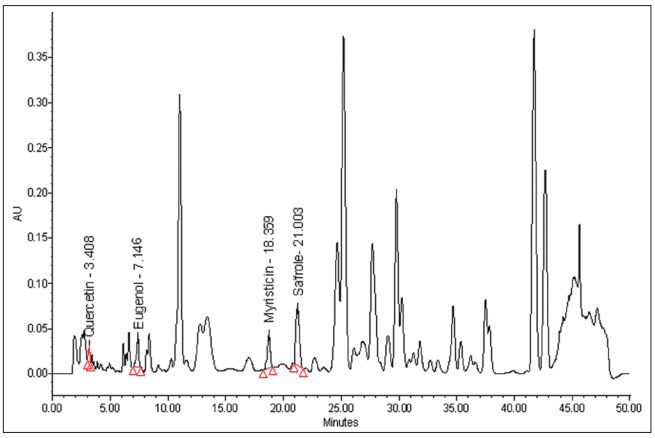

Figure 5: Chromatogram of extract of fruit

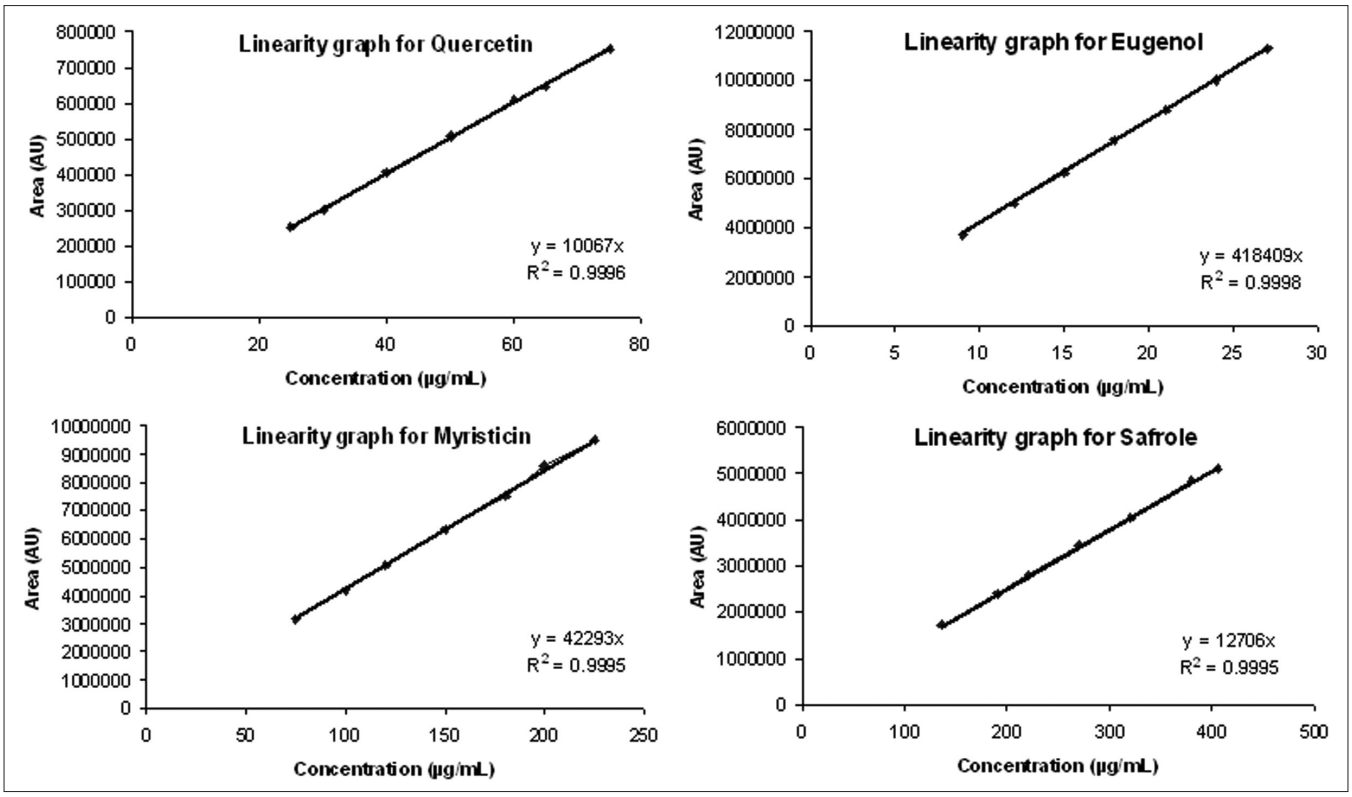

Figure 6: Linearity graphs for standard

and sample solution, and the values obtained in terms of absolute percent difference against initial response as given in Tables 10 and 11.

Analysis of various extracts for determination of % of QUE, EUG, SAFR, and MYRS

The % of each analyte from various extracts of nutmeg fruit and mace obtained is given in Table 14. The results showed interesting differences in the amounts of each analyte present in different extracts of same plant part. The highest % of QUE, EUG, and SAFR was found in the fruit extract of nutmeg, which was 0.5% in the methanolic extract, and 0.20% and 2.93% in the petroleum ether extract of fruit, whereas the highest % of MYRS was found in mace extract of nutmeg, which was 2.87% in the petroleum ether extract of mace.

The fruit extract of nutmeg contained more percentage of SAFR than the mace extract; however, in contrast to that, the percentage of MYRS was more in all extracts of mace as compared with fruit.

Conclusion

The developed RP-HPLC method is precise, specific, accurate, and robust for determination of QUE, EUG, SAFR, and MYRS, along with that it can be used to estimate the amount

Table 14: % Assay for quercetin, eugenol, safrole, and myristicin from various extracts of nutmeg fruit and mace

Part used	Type of extract	QUE (% w/w) (mean±SD)	EUG (% w/w) (mean±SD)	SAFR (% w/w) (mean±SD)	MYRS (% w/w) (mean±SD)
Nutmeg fruit	Petroleum ether	0.28±0.04	0.20±0.07	2.93±0.06	1.61±0.03
	Ethyl acetate	0.21±0.09	0.14±0.10	2.6±0.12	0.98±0.11
	Chloroform	0.19±0.08	0.11±0.11	2.4±0.09	1.01±0.07
	Methanol	0.5±0.04	0.18±0.05	2.7±0.02	1.5±0.04
	Water: Methanol (1:1)	0.05±0.05	0.02±0.09	0.04±0.05	0.035±0.07
Nutmeg mace	Petroleum ether	0.22±0.06	0.15±0.05	2.01±0.02	2.87±0.02
	Ethyl acetate	0.18±0.04	0.11±0.08	1.02±0.10	1.7±0.10
	Chloroform	0.15±0.07	0.10±0.10	0.97±0.07	1.3±0.07
	Methanol	0.35±0.05	0.12±0.07	1.7±0.05	2.5±0.07
	Water: Methanol (1:1)	0.07±0.04	0.018±0.07	0.05±0.07	0.06±0.04

EUG – Eugenol, MYRS – Myristicin, QUE – Quercetin, SAFR – Safrole, SD – Standard deviation

of the active as well as toxic compound preset in the fruit and mace of nutmeg plant. The proposed method can be used for qualitative as well as quantitative analysis of QUE, EUG, SAFR, and MYRS in herbal extracts and also may be useful for standardization purposes in pharmaceutical industries.

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