IJPSR (2022), Volume 13, Issue 12 **(Review Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 22 March 2022; received in revised form, 08 May 2022; accepted, 22 May 2022; published 01 December 2022

VALIDATED SPECTROSCOPIC AND CHROMATOGRAPHIC TECHNIQUES FOR QUALITY CONTROL ANALYSIS OF APIGENIN IN HERBAL MEDICINES

Shweta B. Chougule [1], Arati B. Jivaje [1], V. S. Mannur [1] and Shailendra S. Suryawanshi [*2]

Department of Pharmaceutical Quality Assurance [1], Department of Pharmaceutical Chemistry [2], KLE College of Pharmacy Belagavi, KLE Academy of Higher Education and Research, Belagavi - 590010, Karnataka, India.

Keywords:

Apigenin, Flavonoid, Validation, Quality Control, Herbal Medicines

Correspondence to Author:
Mr. Shailendra S. Suryawanshi

Assistant Professor,
Department of Pharmaceutical Chemistry, KLE College of Pharmacy Belagavi, KLE Academy of Higher Education and Research, Belagavi - 590010, Karnataka, India.

E-mail: shailendrasuryawanshi@klepharm.edu

ABSTRACT: The herbal medicines have been largely used since form several thousands of years in all developing and developed countries in the primary health care of community and society. Flavonoids are the class of polyphenolic secondary metabolites, which are mainly found in plants. Apigenin is a flavonoid which shows several pharmacological activities and used effectively for the management and treatment of several diseases and disorders. Many herbal medicines and their preparation containing Apigenin are available in market and hence Quality Control and standardization of Apigenin is important and essential in ayurvedic industries. The scientific research articles published on analytical development and validation of Apigenin and its estimation in its extract, isolated form and other herbal or polyherbal formulations were reviewed. We have mainly focused on chromatographic and spectroscopic techniques for qualitative and quantitative analysis of Apigenin and presented in this review work. We conclude here that the spectroscopic and chromatographic methods play significant role in the quality control and standardization of Apigenin in herbal medicines.

INTRODUCTION: Herbal medicinal plants have been recognised for millennia as a rich source of therapeutic agent for prevention of diseases and disorders and are highly respected all over the world. Nature has bestowed upon our land an abundance of herbal medicines in the form of plants. Among ancient civilisation, India has been known to be rich storehouse of herbal medicinal plants, treatment with herbal medicinal plants is considered as safe because there is no or least side effects.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.13(12).4857-64

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.13(12).4857-64

More than three- quarters of the world's population depends mainly on herbal medicinal plants for their health care needs. Plant-based medications are becoming more popular in both developed and developing countries as people realise that natural products have fewer side effects, non-toxic and readily available [1]. With the increasing use of herbal goods, a wide range of phytomedines, herbal medicine and their formulation are widely available in the market all around the world.

Increase in demand of herbal medicine has faith that all natural products are safe and effective. However, there are still some concerns are associated with herbal medicine concerning its pharmacognosy, standardization and Quality control of drugs and it has become a public health concern [2]. The herbal drug used in developing

countries of the world lacks official quality control standards, there is no way to ensure that the quality of different batches is consistent. Quality control includes safety of herbal drugs, efficacy and toxicity in herbals and their interactions these quality control parameters need to be tested before the finished product is manufactured, marketed, and ingested by patients. Standardization of herbal formulation is required for the evaluation of high-quality medications. There are variety of tools available that can be used to both qualitative and quantitative measures of herbal medicine. To isolate, identify the phytoconstituents responsible for the medicinal potential of herbs/plants, sophisticated analytical techniques are continually being developed und upgraded. Mainly the spectroscopic and chromatographic methods play major role in quality control and standardization of herbal formulation [3].

Flavonoids are the class of polyphenolic secondary metabolites. They are the important class of natural products, widely observed in flowers, plants, tea, grains, bark, stem, roots, vegetables, fruits and wine. Flavonoids, being the main active ingredient, provide a variety of health-promoting pharmacological effects such as anti-allergic, anti-oxidant, anti-inflammatory, anti-mutagenic and anti-carcinogenic effects. Apigenin is a flavonoid that has been used as a traditional herbal medicine because of its anti-oxidant, anti-inflammatory, antibacterial and antiviral properties. Apigenin is also known for its anticancer properties, which can be used to treat a variety of cancers. It has great demand due to its safety and effectiveness [4].

Apigenin is a flavonoid, widely found in a variety of fruits, vegetables and plants. Vegetables such as parsley, oranges, onions, maize, tea, rice, wheat sprouts chamomile, wheat sprouts. Parsley and peppers are two most common sources of flavones containing Apigenin. Apigenin is a polyphenolic metabolite, chemically known as 4',5,7, - trihydroxy flavone [5].

MATERIALS AND METHODS: Numerous herbal medicines and their preparation containing Apigenin are available in market. Various scientific research articles published in quality control analysis and standardization of Apigenin in its extract, isolated from or any other herbal or polyherbal formulation is being reviewed in the present article, we have mostly focused on the chromatographic and spectroscopic methods. The details of methodological analysis and interpretation of results were presented as below:

Quality Control Analysis of Apigenin in Herbal Medicines by Spectroscopic and Chromatographic Techniques: Numerous research articles, data, and reports are published by scientist on spectroscopic and chromatographic method for quality control and standardization of herbal medicine were presented below and reported as follows:

Spectroscopic Techniques: Arun P. *et. al.,* have reported validation of UV- method for quantification of Apigenin- hydrogenated Soy phosphatidylcholine complex. The UV spectra run in the range of 200-400nm and the maximum wavelength of Apigenin was observed at 335nm and 268nm.

They have used methanol as solvent system. Calibration curve was constructed using concentration range of 2-12µg/ ml at 335nm and 2-14µg/ml at 268nm. The reported method was not economical because they have used high percentage organic phase [6]. Rathode P. *et. al.,* have reported validation of UV- method for quantification of Apigenin in leaves extract of *Parsley.* In this method maximum wavelength of Apigenin was observed at 208nm. They have used methanol as solvent system in ratio of 50:50 % v/v. Calibration curve was constructed using concentration range of 0.5 to 12µg/ml [7].

Shetti P. *et. al.,* have reported optimization of a validated UV-spectrophotometric methodology for assessment of Apigenin in bulk powder. In this method maximum absorbance wavelength of Apigenin was observed at 267nm. They have used methanol as solvent. The detector response for apigenin was linear in the sure concentration range of 2µg/ml-10µg/ml with relation coefficient of 0.9995. This reported method was found to be specific, selective linear, precise, rugged, reproducible for estimation of apigenin with %RSD values less than 2 [8]. The detailed summery of spectroscopic standardization of Apigenin is presented in **Table 1.**

TABLE 1: SPECTROSCOPIC METHODS FOR ANALYSIS OF APIGENIN IN HERBAL MEDICINES

Sl. no.	Author Name	Title of the Work	Description of Analysis
1	Arun P. *et. al.,*	Preparation, Development and Validation of UV-Spectrophotometric Method for the Estimation of Apigenin- Hydrogenated Soy Phosphatidylcholine (HPSC) Complex	The UV spectra run in the range of 200-400nm and the maximum wavelength of Apigenin was observed at 335nm and 268nm
2	Rathod P. *et. al.,*	Development and Validation ofUV- Visible Spectrophotometric Method for Estimation of Apigenin in *Parsley Leaves* Extract	Methanol is used as solvent system in the ratio of 50:50% v/v. Maximum spectra of Apigenin was observed at 208nm
3	Shetti P. *et. al.,*	Optimization of a Validated UV-Spectrophotometric Methodology for Assessment of Apigenin in Bulk Powder	Methanol is used as solvent system. Maximum absorbance wavelength was observed at 267nm

Chromatographic Techniques: Rajgopal S. *et. al.*,have reported reverse phase high- performance liquid chromatography (RP-HPLC) technique for concurrent determination of Apigenin and Luteolin in ethanol extract of *Clerodendrum Serratum* leaves. Method using C18G column (250mm×4.6mm i.d, 5µm) and methanol-acetonitrile- acetic acid- orthophosphoric acid-water (40:20:0.05:0.05:40) as solvent system at flow rate of 0.6mL min^{-1} and detection wavelength at 352nm. LOD was observed 0.05µg/mL for Luteolin along with 0.6µg/ml for Apigenin. LOQ was observed 0.15µ/ml for Luteolin along with 1.8µg/ml for Apigenin [9]. Rajshekaran A. *et. al.*, have reported simultaneous quantification of Luteolin and Apigenin in method leaf extract of *Bacopa monnieri Linn* by HPLC. The separation is achieved on C_{18} column (5µm, 200mm×4.6mm, id) and potassium dihydrogen phosphate buffer (20mM, pH 3-5 modified with ortho phosphoric acid, v/v) and acetonitrile as solvent system with flow rate of 1ml/min and detection wavelength achieved at 348nm.

LOD was established 0.03µg/ml for Luteolin and 0.04µg/ml for Apigenin. LOQ was established 0.91µg/ml for Luteolin along with 0.13µg/ml for Apigenin [10]. Che Zain MS. *et. al.*, have reported ultra-high performance liquid chromatography-ultraviolet/ photodiode array(UHPLC-UV/ PDA) methodfor simultaneous estimation of Luteolin and Apigenin derivatives from leaf extracts of *Elaeis guineensis.* method is carried out using BEH C_{18} column with 1.7µm particle size, 100mm length and 2-1 mm internal diameter for separation and the elution of compound was assisted with two mobile phase water 0.1% formic acid and 0.063% ammonium formate as solvent A and 0.1% formic acid and acetonitrile as solvent B, at flow rate of 0.40mL/min [11]. Subramanian G. *et. al.,* have reported RP-HPLC technique for concurrent quantification of Luteolin and Apigenin from *Achillea millefolium* linn. Method using Hibar Lichrospher C_8 column with ultraviolet detector for separation at a wavelength of 269nm and methanol (20:80 v/v) with 0.5% trifluoracetic acid as solvent systemat flow rate of 1.0ml/min [12]. Gudzenko A. *et. al.,* have reported simultaneous determination of Luteolin and Apigenin in *Achillea Millefolium* L. herb RP-HPLC method. Method using waters x-terra C_{18} column (250×4.6nm i.d, 5µm particle size) for separation. Mobile phase consists of two different solution A & B, both solutions consist of acetonitrile and acetic acid in the ratio of 90:10 for solution A and 10:90 for solution B at flow rate of 1mL/min and detection wavelength at 350nm. The LOD was found to be 0.02µg/ml for Luteolin and 0.02 for Apigenin. The LOQ was observed at 0.05µg/ml for Luteolin along with 0.02µg/ml for Apigenin [13].

Guzelmeric E. *et. al.*, have reported high performance thin layer chromatography (HPTLC)technique for Apigenin 7-O-glucoside in chamomile flowers and its application for fingerprint discrimination of chamomile-like materials. Method using silica gel 60NH2F254s HPLC plate for separation and formic acid-ethyl acetate- acetic acid-water (1.5:30:1.5:3, v/v/v/v) as solvent system [14]. Sharafi A. *et. al.*, have reported HPLC technique for quantification of Apigenin from dried powder of *Cosmos Bipinnatua*, *Apium Graveolens* and *Petroselinum Crispum*. Separation is achieved by using C_8stationary phase (250mm*4.6mm, particle size 5µm; Perfectsill, MZ-Analysentechnik, Germany) provided with a guard column of the same packing. Water and acetonitrile (55:45v/v) as mobile phase with flow rate of 1ml/min at 340nm wavelength the eluted peaks were detected by UV detector. The LOD was

established at 0.005µg/ml. LOQ was established at 0.01µg/ml [15]. Gomathy S. *et. al.,* have reported HPLC technique for the concurrent quantification of Luteolin and Apigenin in commercial preparation. Method using HibarLichrospher C_8 (150×4.6mm i.d., 5µ) for separation. methanol (20:80 %, v/v) with0.5% trifluoracetic acid as solvent system with flow rate of 1ml/min at 269 wavelenght. The developed technique was established linear above the concentration ranging from 10 to 110ng/ml with >0.99 R2. LOD was established 5.0 for Apigenin with 7.0ng/ml for Luteolin. The LOQ was established at 10ng/ml for both Luteolin and Apigenin [16].

Panchal H. *et. al.,* have reported validated HPTLC technique for concurrent quantification of Luteolinand Apigenin in *Achillea millefolium*. As a stationary phase, method uses a silica gel 60 F254 HPTLC plates. Ethyl acetate: formic acid: toluene (4:0.3:6 v/v/v) as solvent system. The determination was accomplished at 254nm using densitometric absorbance-reflection mode for Apigenin and luteolin. The calibration curve was found to be linear between 200-1200ng/spot for Apigenin and 500-3000ng/spot for Luteolin. The LOD was found to be 71.06ng/spot for Apigenin and 74.05ng/spot for Luteolin. LOQ was found to be 230.33ng/spot for Apigenin and 243.72ng/spot for Luteolin [17].

Gtowacki R. *et. al.,* have reported micellar electrokinetic chromatography fordetermination of total Apigenin in herbs. Method using a buffer solution consisting of 30mmol/L sodium borate (pH 10.2), 10% acetonitrile, and 10 mmol/L sodium dodecyl sulfate for separation. The method was validated and calibrated for total Apigenin in the range of 1.0-100µmol/L (R^2 = 0.994). The LOD was found to be 0.48µmol/L and LOQ was found to be 0.92µmol/L[18]. Gomathy S. *et. al.,* have reported RP-HPLC technique for the concurrent estimation of Luteolin and Apigenin from *Achillea millefolium* linn. The estimation is carried out using HibarLichrospher C_8 column (150mm×4.6mm i.d., particle size 5µ) and methanol (20:80 v/v) with 0.5% trifluoroacetic acidas solvent system at a flow rate of 1.0ml/min and detection wavelength of 269nm. The standard curve was constructed using concentration range of 1-5µg/ml with linear correlation coefficient of R^2 = 0.99 [19].

Attarde DL *et. al.,* have reported HPLC technique concurrent quantification of Luteolin and Apigenin in ethyl acetate extract of *Premna integrifolia L*and inselected marketed herbal formulation of dashmula. Method using Pre- coated Silica Gel G $60F_{254}$ HPTLC Plates (0.2mm Thick, Size-20×10cm) as stationary phase and ethyl acetate: toulene: formic acid (4:6:0.15) as mobile phase. Retention factor was 0.39 and 0.29 for standard Apigenin and Luteolin at 347nm iso absorptive wavelength [20]. Satpathy S. *et. al.,* have reported HPTLC method for simultaneous estimation of Apigenin and Luteolin in *Hygrophilia spinosa T anders*.

Method using HPTLC plates for separation and toluene-ethyl acetate-formic acid (6.0:4.0:1.0 v/v) as solvent system and observation was achieved at 349nm. The calibration curve range between 80-560ng/band (R^2 =0.997) for Apigenin and 40-280ng/band (R^2 =0.998) for Luteolin. The LOD was found to be 6.25 for Apigenin and 2.36 for Luteolin. LOQ was found to be 18.95ng for Apigenin and 7.55ng for Luteolin [21]. Patel NG. *et. al.,* have reported HPTLC method for estimation of Luteolin and Apigenin in *Premna mucronate Roxb*., Verbenaceae. Method using precoated silica gel 60 F_{254} HPTLC aluminium plates (10×10cm, 0.2mm thick) as stationary phase and ethyl acetate: formic acid: toluene (4:0.3:6) as mobile phase and detection was achieved at 366 nm.

Calibration curve was found to be linear between 200 and 1000ng/band for Luteolin and 50 and 250ng/band for Apigenin. The LOD was found to be 42.6ng/band for Luteolin and 7.97ng/band for Apigenin. The LOQ was found to be 129.08ng/band for Luteolin with24.155ng/band for Apigenin [22]. Shambhu N. *et. al.,* have reported HPTLC method for simultaneous estimation of Luteolin, Apigenin from *Cardiospermum halicacabum Linn*. and *Hydnocarpus pentadra* (BUCH. -HAM.) OKEN. Method using silica gel $60F_{254}$ TLC plates as stationary phase and methanol: formic acid: chloroform (1.0:2.0:8.0) as mobile phase. The LOD was found to be 0.01µg/band for both Luteolin and Apigenin. The LOQ was found to be 0.02µg/band for Luteolin and 0.03µg/band for Apigenin [23]. Elgin G. *et. al.,* have reported HPLC method for the determination of antioxidant flavonoids of some *Hypericum L.*

Species. Method using C_{18} (ODS) analytical column for separation and methanol and 0.01M orthophosphoric acid (pH 7) (50:50, v/v) as mobile phase. Rutin, Quercitin-3'-glucoside (isoquercitrin), Luteolin-4'glucoside, Quercitin-4'glucoside, Quercitin, Naringenin, Luteolin and Apigenin were chosen as the model compounds for the study [24]. Koneru S. *et. al.,* have reported newly developed analytical method for standardization of Apigenin by RP-HPLC method. Method using Merk C_{18} analytical column (5µm, 250mm× 4.6mm, I.D) and acetonitrile and phosphate buffer at acidic pH (70:30 v/v) as solvent system and observation was achieved at 268nm. Calibration curve was found to be linear between range of 10-50µg/ml with correlation coefficient of 0.9996. Retention time was found to be 3.53min. The LOD was found to be 3.3^{σ}/s and LOQ was found to be 10^{σ}/s [25].

Kim J. *et. al.,* have reported HPLC-UV based quantitative analytical method for Apigenin-7-O-glucoside in *Matricaria chamomilla* extract. Method using Capcell Pak MGII C_{18} (250×4.6mm, 5um) for separation and 20% acetonitrile in aqueous 0.05% (v/v) trifluoroacetic acid as solvent system at a flow rate of 1.0 mL/min and observation was achieved at 330nm. The LOD was established at 0.57mg/ml and LOQ was established at 1.73mg/ml [26]. Watak S. *et. al.,* have reported column chromatography method for isolation of Apigenin from *Trigonella foenum Graecum* seed extract on lab scale. Method using butanol: ethylacetate (1:1) as mobile phase and Silica Gel is used for column packing. The compound isolated from column was evaluated by performing HPTLC, UV, IR and HPLC with standard Apigenin [27]. Parikh N. H. *et. al.,* have reported HPTLC method for estimation of apigenin in *Ocimum basilium L*. Seed (takhmaria). Method using precoated silica gel G60, HPTLC plate for separation and toluene: formic acid: acetone (5:1:4 v/v) as mobile phase and densitometric estimation was done at 340nm. Calibration curve was achieved in the range of 100-600ng and method was found to be linear 0.995 [28].

Khuluk R F *et. al.,* have reported high performance liquid chromatography- diode array detection (HPLC-DAD) method for estimation of flavonoids in *Sonchus arvensis*. The estimation was carried out by using reverse phase C_{18} column and methanol and 0.2% aqueous formic acid as solvent system with flow rate at 1 mL/min. The LOD was observed at 0.006-0.015µg/mL and LOQ was observed at 0.020-0.052µg/mL. The method for the estimation of eight flavonoids that are apigenin, kaempferol, orientin, rutin, hyperoside, myricetin, luteolin and quercetin [29]. The detailed summery of chromatographic standardization of Apigenin is Presented in **Table 2.**

TABLE 2: CHROMATOGRAPHIC ANALYSIS OF APIGENIN IN HERBAL MEDICINES

SI. no.	Author Name	Title of the work	Description of Analysis
1	Rajgopal S. *et. al.,*	Development and Validation of RP-HPLC Method for Simultaneous Determination of Apigenin and Luteolin in Ethanol Extract of *Clerodendrum serratum (Linn.)* Leaves	RP-HPLC method using C_{18}G column (250mm×4.6mm i.d, 5µm) and methanol-acetonitrile-acetic acid-orthophosphoric acid-water (40:20:0.05:0.05:40) as mobile phase
2	Rajsekaran A. *et. al.,*	Simultaneous Estimation of Luteolin and Apigenin in Methanol Leaf Extract of *Bacopa monnieri Linn* by HPLC	The simultaneous estimation carried out using C_{18} column (5µm, 200mm×4.6mm, id) and Potassium dihydrogen phosphate buffer(20mM, pH3-5 adjusted with ortho phosphoric acid, v/v) and acetonitrile as mobile phase
3	Che Zain MS. *et. al.,*	UHPLC-UV/PDA Method Validation for Simultaneous Quantification of Luteolin and Apigenin Derivative from *Elaeisguineensis* Leaf Extract: an Application for Antioxidant Herbal Preparation	UHPLC-UVmethod is carried out using BEH C_{18} column with 1.7µm particle size, 100mm length and 2-1mm internal diameter for separation and the elution of compound was assisted with two mobile phase water 0.1% formic acid and 0.063% ammonium formate as solvent A and acetonitrile and 0.1% formic acid as solvent B
4	Subramanian G. *et. al.,*	Stability Indicating Reverse-PhaseHigh-Performance Liquid Chromatography Technique for the Simultaneous Quantification of Apigenin and Luteolin	RP-HPLC method using HiberLichrospher C_8 column with ultraviolet detector for separation at a wavelength of 269nmand 0.5% trifluoracetic acid along with Methanol(20:80 v/v) as solvent system

		from *Achellia millefolium Linn.*	
5	Gudzenko A. *et. al.,*	RP-HPLC Method forthe Simultaneous Determination of Luteolin and Apigenin In Herb of *Achillea millefolium L.*	RP-HPLC method using waters x-terra C_{18} column (250× 4.6nm i.d, 5µm particle size) as stationary phase. Mobile phase consists of two different solution A & B, both solution consist of acetonitrile and acetic acid in the ratio of 90:10 for solution A and 10:90 for solution B at flow rate of 1mL/min and detection wavelength at 350nm.
6	Guzelmeric E. *et. al.,*	Development and Validation of an HPTLC method for Apigenin 7-O-glucoside in Chamomile Flowers and its Application for Fingerprint Discrimination of Chamomile-Like Materials	HPTLC method using silica gel 60NH2F254s HPLC plate for separation and formic acid-ethyl acetate-acetic acid-water (1.5:30:1.5:3, v/v/v/v) as solvent system [14]
7	Sharafi A. *et. al.,*	Simple and Sensitive High Performance Liquid Chromatography (HPLC) Method for Quantification of Apigenin from Dried Powder of *Cosmos bipinnatua, Apium graveolens* and *Petroselinum crispum*	HPLC method separation is achieved by using C_8stationary phase (250mm*4.6mm, particle size 5μm; perfectsill, MZ-analysentechnik, germany) equipped by a guard column of the same packin. water and acetonitrile (55:45v/v) as mobile phase with flow rate of 1ml/min at 340nm wavelength the eluted peaks were detected by UV detector
8	Gomathy S. *et. al.,*	Development and Validation of HPLC Method for the Simultaneous Estimation of Apigenin and Luteolin in Commercial Formulation.	HPLC method forthe estimation is carried out using HibarLichrospher C_8 (150×4.6mm i.d., 5µ) column for separation. Methanol (20:80 v/v) with 0.5% trifluoroacetic acidas solvent system with flow rate of 1ml/min at 269 wavelenght
9	Panchal H. *et. al.,*	Development of Validated High Performance Thin Layer Chromatography Method for Simultaneous Determination Of Apigenin And Luteolin in *Achillea millefolium*	HPTLC Method using silica gel 60 F254 HPTLC plates as stationary phase. Toluene: Ethylacetate: formic acid (6:4:0.3 v/v/v) as solvent system. The determination was accomplished at 254nm using densitometric absorbance-reflection mode for Apigenin and Luteolin.
10	Gtowacki R. *et. al.,*	Determination of Total Apigenin in Herbs by Micellar Electrokinetic Chromatography with UV Detection.	Method using a buffer solution consisting of 30mmol/L sodium borate(pH 10.2), 10% acetonitrile, and 10 mmol/L sodium dodecyl sulfate for separation
11	Gomathy S. *et.al.,*	Stability-Indicating Reverse-Phase High-Performance Liquid Chromatography Method for the Simultaneous Quantification of Apigenin and Luteolin from *Achillea millefolium Linn*.	RP-HPLC method using HibarLichrospher C_8 column (150mm×4.6mm i.d., particle size 5µ) as stationary phase and methanol and 0.5% trifluoroacetic acid (80:20 v/v) as solvent system at a flow rate of 1.0ml/min and detection wavelength of 269nm
12	Attarde DL *et. al.,*	Validation and Development of HPTLC Method for Simultaneous Estimation of apigenin and Luteolin in Selected Marketed Ayurvedic Formulation of Dashmula and in Ethyl Acetate Extract of *Premna integrifolia L*.	HPTLC method using pre-coated silica gel G $60F_{254}$ HPTLC plates (0.2mm Thick, Size-20×10cm). Ethyl acetate: toulene: formic acid (4:6:0.15) as mobile phase. Retention factor was 0.39 and 0.29 for standard Apigenin and Luteolin at 347nm iso absorptive wavelength
13	Satpathy S. *et. al.,*	Development and Validation of Novel High Performance Thin- Layer Chromatography Method for Simultaneous Determination of Apigenin and Luteolin in *Hygrophilia spinosa T. Anders*	HPTLC method using HPTLC plates for separation and toluene-ethyl acetate-formic acid (6.0:4.0:1.0 v/v) as mobile phase and detection was achieved at 349nm.
14	Patel NG. *et. al.,*	Validated HPTLC Method for Quantification of Luteolin and Apigenin in *Premna mucronate Roxb.,* Verbenaceae	HPTLC method using precoated silica gel $60F_{254}$HPTLC aluminium plates (10×10cm, 0.2mm thick) as stationary phase and ethyl acetate: formic acid: toluene (4:0.3:6) as mobile phase and detection was achieved at 366 nm.

15	Shambhu N. *et. al.,*	Development, Validation of HPTLC Method for Simultaneous Quantification of Luteolin, Apigenin from *Cardiospermum Halicacabum Linn*. and *Hydnocarpuspentandra* (BUCH. -HAM.) OKEN	HPTLC method using Silica gel 60F_{254}TLC plates as stationary phase and methanol: formic acid: chloroform (1.0:2.0:8.0) as mobile phase
16	Elgin G. *et. al.,*	Development and Validation of a Multidetector HPLC Method for the Determination of Antioxidant Flavonoids of Some *Hypericum L.* Species	HPLC method using $C1_8$ (ODS) analytical column for separation and methanol and 0.01M orthophosphoric acid (pH 7) (50:50, v/v) as mobile phase. Rutin, Quercitin-3'-glucoside (isoquercitrin), Luteolin-4'glucoside, Quercitin-4'glucoside, Quercitin, Naringenin, Luteolin and Apigenin were chosen as the model compounds for the study.
17	Koneru S. *et. al.,*	Validation of Newly Developed Analytical Method For Standardization of Apigenin Using RP-HPLC Method in prepared extract.	RP-HPLC method using Merk C18 analytical column (5μm, 250mm× 4.6mm, I.D)and acetonitrile and phosphate buffer at acidic pH (70:30 v/v) as mobile phaseand detection was achieved at 268nm
18	Kim J. *et. al.,*	Development and Validation of HPLC-UV Based Quantitative Analytical Method for Apigenin-7-O-glucoside in *Matricaria chamomilla* Extract.	HPLC-UV Method using Capcell Pak MGII C18 (250×4.6mm, 5um) for separation and 20% acetonitrile in aqueous 0.05% (v/v) trifluoroacetic acid as solvent system at a flow rate of 1.0 mL/min and observation was achieved at 330nm
19	Watak S. *et. al.,*	Isolation of Apigenin from *Trogonella foenum Graecum* Seed Extract Using Column Chromatography on Lab Scale.	Column chromatography method for separation of Apigenin on lab scale using butanol: ethylacetate(1:1) as mobile phase. And silica gel is used for column packing. The compound isolated from column was evaluated by performing HPTLC, UV, IR And HPLC with standard Apigenin
20	Parikh N H *et. al.,*	Development and Validation of High-Performance Thin Layer Chromatographic Densitometric Method for the Quantification of Apigenin in *Ocimumbasilicum L*. Seed (Takhmaria)	HPTLC method using precoated silica gel G60, HPTLC plate for separation and toluene: formic acid: acetone (5:1:4 v/v) as mobile phase and densitometric estimation was done at 340nm
21	Khuluk R F *et. al.,*	An HPLC-DAD Method to Quantify Flavonoids in *Sonchus arvensis* and Able to Classify the Plant Parts and Their Geographical area through Principal component Analysis.	HPLC-DAD method using reverse phase C_{18} column as stationary phase and methanol and 0.2% aqueous formic acid as solvent system with flow rate at 1 mL/min.

CONCLUSION: Quality control analysis is major and essential step in the manufacturing of herbal formulations as the quality of product affects the safety and efficacy of medicinal products. The quality control parameters need to be tested before the finished product is manufactured, marketed, and ingested by patients. Flavonoids are the class of polyphenolic secondary metabolites, which are derived from plants. Apigenin is a flavonoid that has a variety of medicinal properties. Many herbal medicines containing Apigenin are available in market and hence quality analysis is essential. The spectroscopic and chromatographic methods play major role in the quality control and standardization of Apigenin in its extract, isolated from or any other herbal or polyherbal formulation.

ACKNOWLEDGEMENT: Nil

CONFLICTS OF INTEREST: Nil

REFERENCE:

1. Sharma A, Shankar C, Tyagi LK, Singh M and Rao CV: Herbal medicine for Market potential in India. Academic Journal Pf Plant Science 2018; 1(2): 26-36.
2. Woo CSJ, Lau JS and El-Nezami H: Herbal Medicine: toxicity and recent trends in assessing their potential toxic effects. Advance botanical Reseach 2012; 62: 365-84.
3. https://doi.org/10.1007/978-981-10-8291-7_10.
4. Yan X, Qi M, Li P, Zhan Y and Shao H: Apigenin in cancer therapy: anti-cancer effects and mechanisms of action. Cell & Bioscience 2017; 7:50.
5. Ali F, Rahul, Naz F, Jyoti S and Siddique YH: Health functionality of apigenin: a review. International Journal of Food Properties 2017; 20(6): 1197-238.
6. Arun P, Amol T and Darshan T: Preparation, development and validation of UV- spectrophotometric method for the estimation of Apigenin – Hydrogenated soy phosphatidylcholine (HPSC) complex. International Journal of Pharmacy and Pharmaceutical Sciences 2016; 4(3): 220-8.

7. Rathod P, Wakte P and Bhusari SS: Development and Validation of UV- Visible Spectrophotometric Method for Estimation of Apigenin in Parsley Leaves Extract. Int J of Recent Advance in Multid Topics 2021; 2(8): 68-72.
8. Shetti P and Jalalpure SS: Optimization of a validated UV-Spectrophotometric Methodology for Assessment of Apigenin if Apigenin in Bulk Powder. Indian J of Pharma Education and Research 2021; 56(1): 281-86.
9. Rajgopal S, Vazhayil B, Varghese L and Nanjaian M: Development and Validation of RP-HPLC Method for Simultaneous Determination of Apigenin and Luteolin in Ethanol Extract of *Clerodendrum serratum* (Linn.) Leaves. Asian Journal of Applied Sciences 2017; 5(1): 52-60.
10. Rajsekaran A, Sarathikumar N, Kalaiselvan V, Kalaivani M. Simultaneous Estimation of Luteolin and Apigenin in Methanol Leaf Extract of *Bacopa monnieri* Linn by HPLC. British J of Pharma Res 2014; 13(4): 1629-37.
11. Che Zain MS, Osman MS, Yee Lee S and Shaari K: UHPLC-UV/PDA method validation for simultaneous quantification of luteolin and apigenin derivative from elaeisguineensis leaf extract: an application for antioxidant herbal preparation. Molecules Multidisciplinary Digital Publishing Institute (MDPI) 2021; 26(4): 1084.
12. Subramanian G, Meyyanathan SN and Byran G: Stability Indicating Reverse-Phase High-performance Liquid Chromatography Method for the Simultaneous Quantification of Apigenin and Luteolin from *Achillea millefolium* Linn. Asian Journal of Pharmaceutical and Clinical Research 2019; 12(4): 169-72.
13. Gudzenko A: Development and Validation of a RP-HPLC Method for The Simultaneous Determination of Luteolin and Apigenin in Herb of *Achillea millefolium* L. The Pharma Innovation Journal 2013; 2(7): 9-14.
14. Guzelmeric E, Yesilada E and Vovk I: Development and Validation of an HPTLC Method for Apigenin 7-O-glucoside in Chamomile Flowers and its Application for Fingerprint Discrimination of Chamomile-Like Materials. J of Pharm and Biomedical Analysis 2015; 107: 108-18.
15. Sharafi A, Manjili H, Bijani S, Ahmadnia A and Danafar H: Simple and Sensitive High Performace Liquid Chromatographic (HPLC) Method For The Determination of the Apigenin from Dried Powder of Cosmos Bipinnatus, *Apium graveolens* and *Petroselinum crispum*. Iranian Journal of Pharmaceutical Science 2016; 12(2): 21-32.
16. Gomathy S, Narenderan TS, Meyyanathan NS and Gowramma B: Development and Validation of HPLC Method for the Simultaneous Estimation of Apigenin and Luteolin in Commercial Formulation. Journal of Critical Reviews 2020; 7(19): 4785-90.
17. Panchal H, Amin A, Mamta S, Bhatt V. Development of Validated High Performance Thin Layer Chromatography Method for Simultaneous Determination of Apigenin and Luteolin in *Achillea millefolium*. Journal of Chemical and Pharmaceutical Research 2017; 9(11): 39-45.
18. Gtowacki R, Furmaniak P, kubalczyk P and Borowczyk: Determination of Total Apigenin in Herbs by MicellarElectrokinetic Chromatography with UV Detection. Journal of Analytical Method in Chemistry 2016; 2016:3827832.
19. Gomathy S, Meyyanathan NS, Byran G. Stability-Indicating Reverse-Phase High-Performance Liquid Chromatography Method for the Simultaneous Quantification of Apigenin and Luteolin from *Achillea millefolium* Linn. Asian Journal of Pharmaceutical and Clinical Research 2019; 12(4): 169-72.
20. Attarde DL, Pal SC and Bhambar RS: Validation and Development of HPTLC Method for Simultaneous Estimation of Apigenin and Luteolin in Selected Marketed Ayurvedic Formulation of Dashmula and in Ethyl Acetate Extract of *Premna integrifolia* L. Journal of Analytical and Bioanalytical Technique 2017; 8(1):1000343.
21. Satpathy S, Patra A and Ahirwar B: Development and Validation of Novel High Performance Thin- Layer Chromatography Methodfor Simultaneous Determination of Apigenin and Luteolin in *Hygrophilia spinosa* T. Anders. Journal of Planar Chromatography- Modern TLC 2018; 31(6): 437-43.
22. https://doi.org/10.1155/2015/682365.
23. Shambhu N and Dighe V: Development, validation of HPTLC Method for Simultaneous Quantification of Luteolin, Apigenin from *Cardiospermum halicacabum* Linn. and Hydnocar puspentandra (BUCH.-HAM.) OKEN. International journal of Pharmaceutical Sciences. 2014; 6(7): 408-12.
24. Elgin G, Konyalioglu S and Kilinc E: Development and Validation of a Multidetector HPLC Method for the Determination of Antioxidant Flavonoids of Some Hypericum L. Species. Journal of Liquid Chromatography and Related Technologies 2008; 32(3): 432-48.
25. Koneru S, Rao G D and Rao MV B: Validation of Newly Developed Analytical Method for Standardization of Apigenin Using RP-HPLC Method in Prepared Extract. Scholar Research Library 2018; 10(3): 30-6.
26. Kim J, Seo C, Kwon J, Kim W and Kim J: Development and Validation of HPLC-UV Based Quantitative Analytical Method for Apigenin-7-O-glucoside in *Matricaria chamomilla* Extract. Food Supplements and Biomaterials for Health 2021; 1(3): 1-9.
27. Watak S and Patil SS: Isolation of Apigenin from *Trigonella foenum* Graecum Seed Extract Using Column Chromatography on Lab Scale. Asian Journal of Research in Chemistry 2012; 5(5): 600-05.
28. Parikh NH and Kothari CS: Development and validation of high-performance thin layer chromatographic-densitometric method for the quantification of Apigenin in *Ocimum basilicum* L. Seed (Takhmaria). Journal of Planar Chromatography 2016; 29(3): 216-20.
29. Khuluk RH, Yunita A, Rohaeti E, Syafitri UD, Linda R, Wah Lim L, Takeuchi T and Rafi M: An HPLC-DAD Method to Quantify Flavonoids in Sonchus Arvensis and Able to Classify the Plant Parts and Their Geographical Area Through Principal Component Analysis. Multidisciplinary Digital Publishing Institute (MDPI). 2021; 8(2): 12.

How to cite this article:
Chougule SB, Jivaje AB, Mannur VS and Suryawanshi SS: Validated spectroscopic and chromatographic techniques for quality control analysis of Apigenin in herbal medicines. Int J Pharm Sci & Res 2022; 13(12): 4857-64. doi: 10.13040/IJPSR.0975-8232.13(12).4857-64.