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Isolation and Analytical Method Development of Flavonol Glycoside, Quercetin-3-O-β-D-Glucoside: A Review

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Abstract

Quercetin-3-O-β-D-Glucoside (QG) is well known phytoconstituents with antioxidant property that has been studied extensively. QG has been used as marker compound alone or along with other plant molecules for standardization of plant extracts. The article discusses various reported methods for isolation and analytical methods for QG to assist phytochemists in selecting critical parameters for development of new purification technique and analytical methods for QG.

Keywords: Flavonol Glycoside, HPLC, HPLC-MS, Isolation, Quercetin-3-O-β-D-Glucoside

1. Introduction

The major challenge relies for global acceptance of herbal formulations is proper standardization technique. Chromatographic methods such as HPLC, HPTLC, GC, HPLC-MS, HPLC-MS-MS, GC-MS, etc are widely employed for marker-based standardization of herbal extracts/formulations using one or more unique compound/s as marker/s. Qurectin-3-O-Glucoside or Quercetin-3-*O*-β-D-Glucopyranoside (QG) is plant-based flavonol glycoside with anti-oxidant property[1] and used as marker compound alone or in combination for standardization of herbal extracts and formulations. QG is also known as isoquercitrin[2].

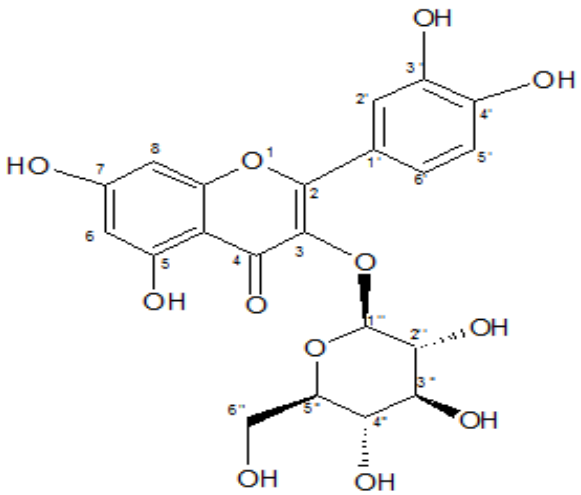

Fig. 1. Structure of Quercetin-3-O-β-D-Glucoside.

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Marker compounds used for herbal standardization are either synthesized or isolated in pure form from corresponding plants by chromatographic techniques. Several attempts have been made for isolation of QG from various medicinal plants. This review presents various described methods for isolation of QG from medicinal plants. The article also focuses on details of various analytical methods for quantitative determination of this phytoconstituent.

2. Physicochemical Parameters and Phytochemistry[3,4]

Molecular formula: $C_{21}H_{20}O_{12}$.

Molecular weight: 464.38 g/mol.

Solubility: The molecule is freely soluble in methanol.

Melting point: 188-190 ^{0}C.

Absorption maxima: 254.8 and 352.6 nm.

Identification: Quercetin-3-O-β-D-glucoside gives orange fluorescence in UV-365 nm after spraying with Natural Products-polyethylene glycol (NP-PEG) reagent.

3. Isolation of Quercetin-3-O-ß-D-Glucoside

A number of methods have been reported for isolation of Quercetin-3-O-β-D-Glucoside from various medicinal plants.

QG was isolated from concord grapes. Grape juice was diluted with distilled water and then passed over an ion exchange column containing Amberlite IRC-50 (H). Elution was carried out using 95% ethanol. The column eluate was evaporated to dryness and extracted with hot anhydrous acetone. The acetone extract was cooled and chromatographed on column containing Magnesol. The column was eluted with ethyl acetate saturated with water to get QG[5].

Aqil *et al* described isolation of QG from roots of *Ricinus communis* by solvent extraction technique and preparative TLC. Dried and powdered roots were defatted using petroleum ether (40-60^{0}C) and then extracted using 80% methanol. The methanol extract was evaporated to dryness and again extracted with petroleum ether. The residue was further extracted with ethyl acetate. Ethyl acetate extract was evaporated to dryness and loaded on silica gel column. Elution was carried out using benzene: Ethyl acetate (5:1, 5:2, 5:4, 5:5, v/v) and ethyl acetate. The ethyl acetate fraction were combined and evaporated and further purified by preparative TLC using chloroform: methanol: water (36.5:13.5: 1.8, v/v/v) as mobile phase to produce pure QG.

Kazuma *et al* isolated QG from petals of *Clitoria ternatea*. Dried petals were extracted with 50% methanol-1% TFA. Non polar compounds were removed by extraction with chloroform and ethyl acetate. The crude extract was separated by MPLC using acetonitrile-water gradient to give two fractions: 20-70% acetonitrile fraction and 70% acetonitrile fraction. The later fraction was loaded on Sephadex LH-20 column and eluted with 5-70% acetonitrile-water gradient to produce three fractions. Fraction produced using 40-50% acetonitrile was again purified by preparative HPLC to produce QG[7].

QG was isolated from dried aerial parts of *Vicia sativa* using solvent extraction technique and column chromatography. The raw material was defatted using petroleum ether and then extracted using 70 % ethanol. Ethanol extract was concentrated and fractionated using polyamide column and ethanol-water eluent. The fractions collected using 60-70% ethanol were combined and further purified by paper chromatography using butanol: Acetic acid: water (4:1:5, v/v/v) as mobile phase to give pure compound[8].

QG was isolated and purified from flowers of *Forsythia viridissimma*. Dried flowers were extracted with 50% methanol. The methanol-water extract was evaporated to dryness. The dry extract was dissolved in hot water and kept for 24 h in refrigerator to get yellow precipitate. The precipitate was subjected to column chromatography using Sephadex LH 20 as stationary phase. Elution was carried out using methanol to get QG[9].

Manguro *et al* isolated QG from methanol extract of leaves of *Embelia keniensis*. Dried leaves were extracted using methanol. Methanol extract was evaporated under reduced pressure and loaded on silica gel column. Elution was carried out sequentially with gradient elution using dichloromethane, dichloromethane and methanol and finally with methanol to get four fractions. Second fraction was further purified by repeated flash chromatography using dichloromethane: Methanol (4:1, v/v) as eluent to give QG[10].

Methanol extract of aerial parts of *Asperula arvensis* was used for isolation of QG. Dried plant material was extracted with methanol and methanol extract was evaporated to dryness. The dry extract was suspended in water and partitioned with chloroform. The aqueous layer was loaded on Sephadex LH 20 column and elution was carried out using methanol to produce six fractions. Third fraction was separated by preparative TLC using chloroform-methanol-water (61:32:7, v/v/v) as mobile phase to give seven fractions. Fourth fraction was again separated by preparative TLC using ethyl acetate: formic acid: acetic acid: water (100:11:11:27, v/v/v/v) to give QG[11].

High Speed Counter Current Chromatography (HSCCC) was used to isolate QG from *Hypericum japonicum*. The raw material was refluxed using 40% ethanol and filtered. The residue was again extracted with 95% ethanol. The resulting alcoholic solution was evaporated to dryness and the dried extract thus obtained was subjected to HSCCC. The solvent systems used were ethyl acetate: ethanol: water (5:1:5, v/v/v) and n-hexane: Ethyl acetate: Ethanol: Water (1:1.2:1.2:1, v/v/v/v). The apparatus was rotated at 800 rpm. The effluent of the column was monitored at 280 nm. Fractions were collected and monitored by HPLC[12].

QG was isolated from berries of *Hippophae rhamnoides*. Berries were mashed and treated with pectolytic enzymes for 1-2 h at 52^0C and juice was separated and clarified with bentonite. The juice was concentrated and treated with n-hexane to remove fatty material. Then the juice was extracted successively with dichloromethane and ethyl acetate. Ethyl acetate extract was evaporated to dryness and subjected to HSCCC. The solvent system used was *n*-hexane: *n*-butanol: Water (1:1:2, v/v/v). The effluent of the column was monitored at 280 nm. Fractions were collected and monitored by TLC. Fractions obtained by HCCC were further purified by reversed phase cartridges. The elution was carried out using methanol: water (30:70, v/v)[13].

Ethanol extract of stems of *Pterogyne nitens* was used for isolation of QG by Regasini *et al*. The plant material was defatted with n-hexane and extracted with ethanol. Ethanol extract was concentrated and dispersed in methanol: Water (4:1, v/v) and then successively partitioned with ethyl acetate and *n*-butanol. The ethyl acetate fraction was subjected to gel permeation chromatography using Sephadex LH-20 and eluted with methanol. Fractions were collected and checked by TLC. Subfractions were further purified by repeated column chromatograhy using silica gel as stationary phase and chloroform: Methanol (3:1, v/v) as eluent to yield pure Quercetin-3-O-β-Glucoside[14].

QG was purified from aerial parts of *Orostachys japonicas*. Dried and powdered aerial parts were extracted with 80 % methanol. The methanol extract was partitioned successively in water, dichloromethane and n-butanol. Butanol fraction was subjected to column chromatography using Lichoprep RP-18 column and elution was carried out using water and methanol. Fraction obtained by water: methanol (4:1, v/v) was loaded on silica gel column and elution was carried out using dichloromethane: Methanol: Water and yielded to 15 subfractions. Ninth subfraction gave pure QG[15].

QG was isolated from aerial parts of *Prangos ferulaceae*. Dried and powdered aerial parts were extracted successively with n-hexane, dichloromethane and methanol. The methanol extract was evaporated to dryness. This residue was fractionated on an SPE-C18 cartridge using different methanol-water mixtures. The fraction obtained by 40% methanol was further purified by preparative HPLC using methanol and water gradient. The retention time of QG was 24.5 min[16].

QG was isolated from aerial parts of *Commelina communis*. The plant material was refluxed with 70 % ethanol. The extract was chromatographed on a Diaion HP-20 column. After washing the column with water, the column was eluted with methanol. The fraction was collected and evaporated to dryness. The residue was loaded on a silica gel column and elution was carried out using dichloromethane: Water. Each fraction was subjected to reversed phase preparative HPLC using acetonitrile: 0.1 % acetic acid (12:88 or 15:85, v/v) as mobile phase to get pure QG[17].

QG was isolated from leaves of *Scutia buxifolia*. The plant material was macerated at room temperature using 70% ethanol. Ethanol extract was evaporated to dryness and suspended in water. The water extract was partitioned successively with dichloromethane, ethyl acetate and *n*-butanol. Ethyl acetate extract was loaded on silica gel 60 column and eluted with various proportions of dichloromethane and ethanol. Fractions were collected and analysed by TLC. Fractions showing

similar TLC profiles were pooled together. Fractions 8 to 28 were rechromatographed on silica gel 60 column and eluted with chloroform and ethanol in various proportions to produce pure QG[18].

QG was isolated from leaves of *Sambucus ebulus* by extraction using methanol. Methanol extract was dried under reduced pressure. Dried methanol extract was loaded on polyamide column and eluted with gradient of methanol–water to get five fractions. Fourth fraction was rechromatographed on LiChroprep RP-18 column and elution was carried out using various proportions of methanol-water to give six subfractions. The second subfraction was further purified on Sephadex LH-20 column and elution was carried out using gradient of chloroform–methanol–water to yield pure QG[19].

QG was isolated from leaves of *Ceratonia siliqua*. Dried and powdered plant material was extracted using 70% ethanol and evaporated to dryness under reduced pressure. The ethanol extract was loaded on Sephadex LH-20 column and elution was carried out using water followed by water-methanol mixtures to produce five fractions. First fraction was purified by column chromatography using polyamide column as stationary phase and water-methanol mixtures in decreasing polarity as mobile phase. Further purification was carried out using paper chromatography using 15 % acetic acid as mobile phase to get QG[20].

QG was isolated from aerial parts of *Diplotaxis harra*. Dried and powdered plant material was defatted using petroleum ether (60–80^{0}C) and extracted with 95% ethanol. Ethanol extract was evaporated to dryness, diluted with water and fractionated successively with chloroform, ethyl acetate and n-butanol. Butanol fraction was subjected to preparative TLC and paper chromatography using ethyl acetate: Methanol: Water (30:5:4, v/v/v) as mobile phase to produce five fractions. These fractions were treated with Methanol: Water (1:1, v/v). Further purification was carried out by reversed phase HPLC using gradient elution with 0.05% formic acid and acetonitrile. Compound obtained from HPLC were subsequently purified by column chromatography using Sephadex LH-20[21].

QG was isolated from leaves of *Azadirachta indica*. Dried and powdered leaves were macerated in ethanol. The resulting ethanol extract was concentrated and extracted successively with n-hexane, ethyl acetate and n-butanol. Ethyl acetate extract was subjected to vacuum liquid chromatography over silica gel. Elution was carried out using solvents in increasing polarity. Fraction obtained by 20% ethanol in ethyl acetate was subjected to gel filtration chromatography using chloroform-methanol mixtures in various proportions to give 30 subfractions. Subfractions 6-16 were combined and further purified by column chromatography using 20% methanol in ethyl acetate to yield pure QG[22].

QG was isolated from *Argyreia nervosa*. The plant material was defatted using (60-80^{0}C) and then extracted with 70% alcohol. The hydroalcoholic extract was evaporated to dryness and subjected to column chromatography. The elution was carried out using pet ether, chloroform, ethyl acetate, methanol and water. Chloroform: ethyl acetate (80:20, v/v) and ethyl acetate: methanol (20:80, v/v) produced QG[23].

QG was isolated from leaves of *Podocarpus gracilior*. The plant material was extracted with hot 80 % methanol. The dry residue of extract was again extracted with chloroform. The chloroform insoluble portion was fractionated on a polyamide column using water and mixture of water and methanol as eluent to get four main fractions. Third fraction was loaded on cellulose column using water-methanol mixtures in decreasing polarity. Fractions thus obtained were purified by repeated column chromatography using Sephadex LH-20 to get QG[24].

QG was isolated from flowers of *Bombax ceiba*. Dried flowers were extracted successively with 70% methanol and pure methanol. Both extracts were evaporated to dryness and suspended in water. The water soluble fraction was subjected to MCI gel CHP20P column chromatography to produce nine fractions. Eighth fraction was loaded on Sephadex LH-20 column and eluted with methanol to get seven subfractions. Fourth subfraction loaded on Sephadex LH-20 column to produce QG[25].

QG was isolated from leaves of *Melia azedarach*. Dried and powdered leaves were extracted at room temperature using 80% methanol. The extract was evaporated and loaded on silica gel column and eluted successively with hexane, chloroform, ethyl acetate and methanol. The methanol fraction was evaporated and loaded on silica gel column and eluted with ethyl acetate and methanol in increasing polarity. Fractions were collected and

monitored by TLC. Fractions showing similar profiles were pooled together and five fractions were obtained. Third fraction (obtained by ethyl acetate: methanol, 1:1, v/v) was reloaded on Sephadex LH 20 and eluted with methanol to get three subfractions. Third subfraction was purified further by column chromatography using Sephadex LH 20 to get pure QG[26].

4. HPLC Analysis of Quercetin-3-O-ß-D-Glucoside

A number of HPLC methods have been reported for quantification of QG alone or in combination with other constituents from various medicinal plants.

QG was determined in *Ranunculus* species by HPLC using Hypersil Ultrabase C18 UB column (250 mm X 0.22 mm) as stationary phase and 2 % acetic acid and acetonitrile in gradient mode was used as mobile phase at the flow rate 0.65 ml/min. The detection was carried out at 340 nm.

Retention time of Quercetin-3-O-β-D-Glucoside was 65.6 min[27].

QG was quantitated in mango by HPLC using LiChrospher C18 column (250 mm X 4 mm, 5 µm) as stationary phase and binary phase consisting of solvent A (2% acetic acid) and solvent B (acetonitrile: 0.5 % acetic acid, 50: 50, v/v) in gradient mode was used as mobile phase at the flow rate 1.0 ml/min. Column oven temperature was set at 25^{0}C. Injection volume was 10 µl. The detection was carried out at 370 nm. Retention time of QG was 32.27 min[28].

QG was quantitated in onions by HPLC using Waters µBondapak C18 column (300 mm X 4.6 mm, 5 µm) as stationary phase and binary phase consisting of solvent A (water: Tetrahydrofuran: TFA, 98: 2: 0.1, v/v/v) and solvent B (acetonitrile) in gradient mode was used as mobile phase at the flow rate 1.0 ml/min. Injection volume was 50 µl. The detection was carried out at 362 nm. Retention time of QG was 9.55 min. The developed method was found to be linear over the range 0.00-87.5 µg/ml[29].

Reversed phase HPLC method was developed for determination of QG in various polyphenolic families using Luna C18 column (150 mm X 4.6 mm, 3 µm) as stationary phase and 0.5% acetic acid and methanol in gradient mode as mobile phase at low rate of 0.8 ml/min. Injection volume was 50 µl. Column oven temperature was set at 30^{0}C. The detection was carried out at 370 nm. Retention time of QG was 81.68 min[30].

Simultaneous determination of five bioactive flavonoids including QG was carried out in *Hypericum japonicum* by HPLC using Luna C18 column (250 mm X 4.6 mm, 5 µm) as stationary phase and methanol and 0.5% acetic acid in gradient mode as mobile phase at flow rate of 1.0 ml/min. Detection was carried out at 350 nm. Column oven temperature was set at 25^{0}C. Retention time of QG was 22.3 min. The method was found to be linear over the range 4.60-94.7 µg/ml. LOD was found to be 25 ng/ml[31].

QG was quantified by HPLC using Gemini C18 110A column as stationary phase and binary phase consisting of solvent A (10 % formic acid) and solvent B (acetonitrile: Water: Formic acid, 50:40:10, v/v/v) in gradient mode was used as mobile phase at flow rate of 1.0 ml/min. The column temperature was set to 40^{0}C. The detection was carried out at 360 nm. Retention time of QG was 40.73 min[32].

HPLC method was developed for detection of QG in aqueous extract of *Lithrea molleoides* using Gemini C18 column (150 mm X 4.6 mm, 5 µm) as stationary phase and binary phase consisting of solvent A (2% acetic acid) and solvent B (2% acetic acid in methanol) in gradient mode was used as mobile phase at flow rate of 1.2 ml/min. Injection volume was 20µl. Retention time of QG was 25.7 min[33].

HPLC method was developed and validated for simultaneous estimation of quercetin glycosides from *Saussurea grandifolia* using Capcell Pak C18 column (250 mm X 4.6 mm, 5 µm) as stationary phase and binary phase consisting of solvent A (0.05% TFA) and solvent B (0.05% TFA in Methanol:Acetonitrile, 60:40, v/v) in gradient mode at flow rate of 1.0 ml/min. The column oven temperature was set at 40^{0}C. Retention time of QG was approximately 23.0 min. The method was found to be linear over the range 1.56-50.0 µg/ml for Quercetin-3-O-β-Glucoside. LOD and LOQ were found to be 0.11 µg/ml and 0.36 µg/ml, respectively[34].

Total flavonoids contents were determined by HPLC using Hypersil BDS C18 column (150 mm X 4.6 mm, 5 µm) as stationary phase and 1% acetic acid and methanol in gradient mode was used as mobile phase at flow rate of 1.0 ml/min. Analysis was carried out at ambient

temperature. The injection volume was 20 µl. Detection was carried out at 334 nm[35].

5. HPLC-MS Analysis of Quercetin-3-O-ß-D-Glucoside

Many HPLC-MS methods have been reported for quantification of QG.

QG was quantitated in alcoholic extract of *Hypericum perforatum* by HPLC-MS. HPLC profile was developed using RP C18 column (250 mm X 4.0 mm, 5 µm) as stationary phase and binary phase consisting of solvent A (0.1% formic acid) and solvent B (methanol) in gradient mode was used as mobile phase at the flow rate 1.0 ml/min. Mass analysis was carried out using ion-trap mass spectrometer. The heated capillary and voltage were maintained at 350^{0}C and 4 kV, respectively. The nebulizer pressure and flow rate of nitrogen were 65.0 psi and 11 l/min, respectively. Retention time of Quercetin-3-O-β-D-glucoside was 18.3 min[36].

Flavonol glucosides were determined in *Allium* species by HPLC-MS technique. The HPLC profile was developed using Discovery C18 Supelco column (250 mm X 4.6 mm, 5 µm) as stationary phase and gradient elution was carried out using acetonitrile and water at flow rate of 1.0 ml/min. Column oven temperature was 30^{0}C. Injection volume was 20 µl. Detection was carried out at 350 nm. For mass analysis, capillary temperature was set at 250^{0}C. Spray needle voltage was set at 4.50 kV. Electron spray capillary voltage was set at +3 V and -47 V for positive and negative polarity, respectively. Retention time of QG was 17.2 min. LOD was found to be 0.4 mg/kg[37].

HPLC-MS method was developed for estimation of phenolic compounds from cultivated strawberries. HPLC profile was developed using Eclipse XDB C18 column (150 mm X 4.6 mm, 5 µm) as stationary phase and 1% formic acid and methanol in gradient mode was used as mobile phase. The flow rate of mobile phase was 0.4 ml/min and injection volume was 20 µl. The column oven temperature was set at 40^{0}C. Detection was carried out at 360 nm. For mass analysis, Nebulizing gas (N_2) pressure and flow rate were 50 psi and 12 l/min, respectively. Temperature of capillary was set at 325^{0}C. Capillary voltage was set at 3.5 kV. Retention time of QG was 32.9 min[38].

HPLC-MS method was developed for identification of chemical constituents from leaves of *Apocynum venetum*. HPLC profile was developed using Agilent C18 column (250 mm X 4.6 mm, 5 µm) as stationary phase and acetonitrile and 0.5% acetic acid in gradient mode as mobile phase at flow rate of 1.2 ml/min. Column oven temperature was set at 25^{0}C. Detection was carried out 254 nm. For mass analysis, sheath gas flow rate was set at 60 bar and auxiliary gas flow rate was set at 10 bar. Electrospray voltage of the ion source and capillary voltage were set at 5 kV and 10 V, respectively. Capillary temperature was maintained at 260^{0}C. Retention time of QG was 28.24 min[39].

HPLC-MS method was developed for determination of flavonoids in Apiaceae family. HPLC profile was developed using Phenomenex C18 column (250 mm X 4.6 mm, 5µm) as stationary phase and binary phase consisting of solvent A (0.5% Acetic acid: Methanol, 60:40) and solvent B (0.5% Acetic acid in methanol) in gradient mode at flow rate of 0.5ml/min. The injection volume was 10 µl. The column oven temperature was set at 25^{0}C. For mass analysis, an electrospray ionization source in the positive mode was used with capillary voltage of 3.5 kV. Retention time of QG was 9.65 min[40].

HPLC-MS method was developed for characterization of flavonoids in 70% ethanol extracts of *A. venetum* leaves and *P. hendersonii* leaves. HPLC profile was developed using Inertsil ODS-3 column (250 mm X 4.6 mm, 5µm) as stationary phase and 0.1% formic acid and acetonitrile in gradient mode was used as mobile phase at flow rate of 1.0 ml/min. The column oven temperature was set at 35^{0}C. Detection was carried out at 360 nm. For mass analysis, the capillary voltage, Curved Desolvation Line (CDL) voltage, and detector voltage were set at 4.5 kV, 10 V and 1.7 kV, respectively. The Nebulizing gas (N_2) was maintained at a flow rate of 1.5 l/ min. Retention time of QG was 47.021 min. The method was found to be linear over the range 10.75-96.77 µg/ml for QG. LOD and LOQ were found to be 60.08 ng/ml and 200.28 ng/ml, respectively[41].

HPLC-MS method was developed for assessment of the uptake and lifespan effects of Quercetin-3-O-β-D-Glucoside and Quercetin in *Caenorhabditis elegans*. HPLC profile was developed using Ascentis RP-Amide column (150 mm X 2.1 mm, 3 µm) as stationary phase and 0.1% formic acid and acetonitrile in gradient mode was used as mobile phase at flow rate of 0.2 ml/min. The column oven temperature was set at 30^{0}C. Detection was carried out at 370 nm. For mass analysis, the ion spray voltage was set

at −4500 V in the negative mode. Declustering potential, entrance potential, collision energy and cell exit potential were set at -40 V, -10 V, -50 V, and - 3 V, respectively. Retention time of QG was 12.6 min[42].

QG was characterized from *Fagopyrum tataricum* using HPLC/PDA/linear ion trap FTICR hybrid mass spectrometry. HPLC chromatogram was generated using Kinetex C18 column (100 mm X 4.6 mm, 2.6 µm) as stationary phase and 0.1% acetic acid and acetonitrile in gradient mode as mobile phase at flow rate of 0.5 ml/min. Injection volume was 2 µl. The column oven temperature was set at 35^{0}C. The detection was monitored simultaneously at 210 nm, 280 nm and 320 nm. For mass analysis, ion spray voltage was kept at 3.5 kV. Capillary temperature was maintained at 250^{0}C. Capillary voltage was set at 30 V and tube lens offset voltage was set at 90 V. Sheath gas flow rate, auxiliary gas flow rate and sweep gas flow rate were adjusted to 45, 10 and 3, respectively. Retention time of QG was 29.66 min[43].

QG was quantified along with five flavonoids from *Paulownia tomentosa* flower extract in rat plasma by LC-MS/MS. Formononetin was used as the internal standard. Acetonitrile and 2mM ammonium acetate were used in gradient mode as mobile phase. Retention time of QG was 2.94 min. For mass analysis, the drying gas temperature and the heater temperature were maintained at 300^{0}C and 350^{0}C, respectively. The Nebulizing gas (N_2) pressure and capillary voltage were maintained at 45 psi and −4.0 kV[44].

HPLC-DAD-MS/MS analysis was carried out for QG with phenolic compounds in ethanolic extract of *Securigera securidaca* flowers. Chromatographic separation was performed on C18 column (50 mm X 2.1 mm, 1.8 µm) using methanol and 0.2% formic acid in gradient mode at flow rate of 0.8 ml/min as eluent. QG eluted with mean retention time of 17.9 min. For MS analysis, capillary voltage and end plate voltage were kept at 4000 V and −500 V, respectively. The nebulizing gas (nitrogen) was maintained at 35 psi. The flow of drying gas was 10 l/min at 350^{0}C[45].

6. Discussion

Many methods are reported for isolation of QG from various plant parts such as fruits, stems, roots, flowers, aerial parts and leaves. Initially extraction was carried out using polar solvents such as methanol and ethanol as QG is polar in nature. Fatty material of the extract was removed either by hexane or by petroleum ether. Column chromatography was method of choice for further fractionation and separation using different combination of solvents as eluent. Few researchers also employed solid phase extraction technique, HSCCC, paper chromatography and preparative HPLC.

For quantitative estimation of QG, reversed phase LC method with gradient mode was preferred. Though most of the HPLC method used strong solvent like acetonitrile as one of the mobile phase component, the mean retention time of QG was high (except methods proposed by Peffley *et al*, Lapcik *et al* and Zhang *et al*).

7. Conclusion

Isolation and purification of plant-based markers involve long laborious extraction procedures and large amount of solvents. The major challenge for such procedures is yield as well as purity of the isolate. Development of analytical methods for phytomarkers is also challenging as they require lengthy chromatographic run times and resolution of closely related components. The present review covers outline of various isolation techniques and HPLC/HPLC-MS methods used in quantitative determination of QG in different medicinal plants. The procedures discussed for isolation and analytical methods involve application of various variables such different chromatographic techniques, stationary phases and mobile phases. The present review would assist the phytochemist in choosing appropriate solvent for isolation of this molecule from another plant. The review would also help in selecting key chromatographic parameters for development of analytical method for QG in plant extracts or in formulations by comparison of available records. New simple and cost effective isolation technique should be adopted which increases yield and purity of QG. Focus should be also be made on development of fast HPLC methods for quantification of QG as the existing one are time consuming.

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