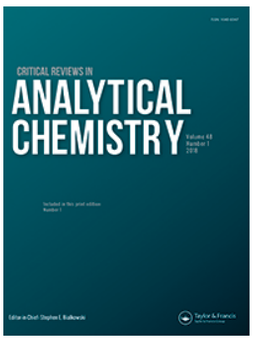

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Characteristics, Biological Properties and Analytical Methods of Ursolic Acid: A Review

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ABSTRACT

Ursolic acid (UA) is a naturally occurring triterpenoid which is a promising candidate for the development of new therapeutic approaches and for the prevention and treatment of several diseases owing to its pharmacological importance. However, its low solubility in aqueous medium affects its therapeutic application. Several strategies have been used to overcome this obstacle. In this study, the incorporation of UA in to different drug delivery systems was found to be highly efficient. In addition, important investigations were performed about methods for qualitative and quantitative analyses of UA in various raw materials, including plants, biological fluids, and drug delivery systems, were investigated. Most recently high performance liquid chromatography coupled with various detectors, gas chromatography-mass spectrometry and capillary electrophoresis were used for this purpose. Thus, this review was performed to evaluate the biological effects of UA demonstrated thus far as well as the currently used, delivery systems and analytical methods.

KEYWORDS

Ursolic acid; biological effects; drug delivery systems; analytical methods

1. Introduction

Ursolic acid is a pentacyclic triterpenoid ($C_{30}H_{48}O_3$; Figure 1) of molecular weight with a 456.68 g/mol and melting point of 283–285°C, which belongs to class of C_{30} isoprenoid compounds. These compounds are widely distributed in nature; therefore, UA can be isolated from various plants, such as *Calluna vulgaris* (heather), *Rosmarinus officinalis* (rosemary), *Callendula officinalis* (marigold), *Melaleuca leucadendron* (melaleuca), *Malus domestica* (apple), *Origanum vulgare* (oregano), *Salvia officinalis* (sage), *Origanum majorana* (marjoram), *Lavandula angustifolia* (lavender flowers), *Thymus vulgaris* (thyme) and *Eucalyptus* (eucalyptus).[1,2] The presence of UA in most medicinal herbs and fruits makes it a component of human diet.[3]

The biosynthesis of UA and other isoprenoid compounds found in plant tissues, occurs from the cyclization of squalene into (3S)-oxidosqualene, which can produce over 80 different carbon skeletons[4] through the activity of oxidosqualene cyclase enzymes that realize the carbocation rearrangements responsible for this biological variety. The common precursor (3S)-oxidosqualene is converted into the dammarenyl ring that undergoes ring expansion and other cyclization reactions to form the characteristic fifth ring present in lupeol, α-amyrin, and β-amyrin skeletons. The α-amyrin represents the UA skeleton, whereas its C30 isomer, β-amyrin is the representative skeleton of the oleanolic acid (OA).[5]

The crystalline solid compound, chemically known as 3β-hydroxy-urs-12-en-28-oic acid, has been used for a long time in folk medicine for its therapeutic activities.[2] Recently, the search for biologically active substances from natural sources has increased interest in its investigation.

Studies indicate that UA has various pharmacological effects, including anti-inflammatory, hepatoprotective, antitumor, cardioprotective, neuroprotective, antimicrobial, antihyperlipidemic, anti-diabetic, antifungal, antiviral and trypanocidal.[6]

However, it is classified as a class IV drug in the Biopharmaceutics Classification System owing to its limited pharmacological effects due to low solubility in water and difficulty in permeating biological membranes.[7] Thus, new approaches have been introduced to enhance the biopharmaceutical properties of this compound, particularly drug delivery technologies. Several UA delivery systems have been used successfully, such as nanoemulsions,[8] mesoporous silica nanoparticles,[9] solid lipid nanoparticles,[10] liposomes,[11] niossomal gels[12] and solid dispersions.[13]

Owing to its numerous important pharmacological effects, analytical methods for UA being increasingly researched. A few studies have reported methods developed for UA analysis. In this study, we reviewed the biological effects of UA and the delivery systems and analytical methods used.

The Scopus, Science Direct and PubMed databases were used for the review. The main descriptors used were as follows: "ursolic acid" in combination with "biological effects", "anti-inflammatory", "antitumor", "drug delivery system", "analytical methods", "HPLC", "GC", "MS", "UPLC", and "electrophoresis".

2. Pharmacological aspects of ursolic acid

Anti-inflammatory effect

Nuclear factor kappa B (NF-kβ) is major cell signaling pathway responsible for the regulation of inflammatory

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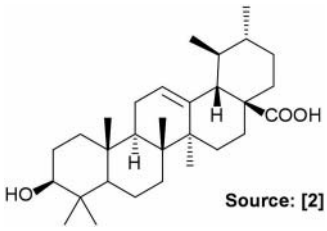

Figure 1. Chemical structure of ursolic acid.

genes expression.[14] The evaluation of anti-inflammatory effects of UA by exposing BALB/c mice to ovalbumin for asthma induction revealed that UA inhibits NF-κB, and thus can be used in the treatment of asthma.[15] Checker et al.[16] observed that UA exhibited anti-inflammatory activity in a mouse model by inhibiting and secretion of cytokines by T cells, B cells, and macrophages, through suppression of the transcription factors NF-κB, nuclear factor of activated T cells, and activator protein-1.

Antitumor effect

In a study, UA showed potential in the prevention and treatment of cancer by influencing several cell signaling enzymes and protecting against carcinogenic agents.[17] The treatment of breast cancer cells with UA at low doses (5–20 μM) caused a G21 / G1 cell cycle arrest, an increase in p21 levels, oxidative stress, and DNA damage at 20 μM, UA induced autophagy and apoptosis of breast cancer cells without normal cell damage.[18] Furthermore, UA enhanced phototoxicity of ultraviolet to visible light broadband radiation by modulating the activation of p53 and NF-κB in melanoma cells.[19]

Hepatoprotective effect

The hepatoprotective effect of UA alone or in combination with other active ingredients on acute and chronic liver injury is well known.[20] Gutiérrez-Rebolledo et al.[21] revealed that the subcutaneous administration of a mixture of UA and OA in male BALB/c mice with hepatic injury induced by anti-tubercular agents resulted in a decrease in aspartate transaminase and alanine aminotransferase levels and improvement in histological alterations. UA inhibited the progression of non-alcoholic fatty liver disease, with a decrease in liver weight and hepatic steatosis, as well as accumulation of intracellular lipids, possibly due to an increase in lipid β-oxidation and inhibition of hepatic stress.[22]

Cardioprotective effect

Cardiovascular disorders are one of the major causes of mortality and morbidity worldwide. UA is effective in the treatment of cardiovascular diseases. Liobikas et al.[23] studied the effect of UA on mitochondria isolated from mouse heart, and investigated whether this compound induced the decoupling of oxidative phosphorylation in the mitochondria without affecting the respiration rate, besides inhibiting H_2O_2 production. In addition, the use UA in the treatment of myocardial fibrosis and hypertrophy in experimental models in vitro and in vivo showed the attenuation of fibrosis and cardiac hypertrophy, possibly through the inhibitory mechanism of the miR-21 / ERK signaling pathways in the myocardial cells.[24]

Neuroprotective

Some studies have shown the efficient neuroprotective effect of UA. It can regulate inflammatory responses in the ischemic brain of rats, which was indicated by a significant reduction in infarct size and a decrease in lipid peroxidation through the activation of the nuclear factor-erythroid 2-related factor 2 (Nrf2).[25] In the study performed by Ding et al.,[26] the use of UA for the treatment of brain lesions in a murine model significantly improved cerebral edema as well as neurological insufficiencies caused by trauma along with a decrease in oxidative stress. This study revealed that the neuroprotective effect of UA is associated with the activation of the Nrf2 pathway.

Antimicrobial properties

In a study by Singh et al.[27] UA exhibited antimycobacterial activity against *Mycobacterium smegmatis, Mycobacterium tuberculosis*, and clinical isolates of multi-drug-resistant *M. tuberculosis* and, clinical isolates of multi-drug-resistant *M. tuberculosis* at a minimum inhibitory concentration (MIC) of 62.5 μg/mL.

Nascimento et al.[28] investigated the effect of UA and its derivatives on the susceptibility of certain pathogenic bacteria to antibiotics belonging to the aminoglycosides class (neomycin, amikacin, kanamycin and gentamicin). 3β-Formyloxy-urs-12-en-28-oic acid (64 μg/mL) in combination with kanamycin showed a synergistic effect against *E. coli*, reducing MIC from 128 μg/mL to 8 μg/mL.

Antihyperlipidemic effect

A few studies on the antihyperlipidemic effect of UA have been carried out. The use of UA and artesunate in the treatment of hyperlipidemia in rabbits revealed that the triglycerides were reduced by the two compounds individually. However, when used together, they significantly reduced cholesterol and triglyceride levels as well as hepatic steatosis and aortic root lesions.[29]

Antidiabetic properties

The antidiabetic property of UA was investigated *in vivo* experimental models for type 2 diabetes. The hepatic insulin resistance was improved by the modification of fatty acid, TNF-α, and adiponectin levels, which enhanced PPARα expression and consequently the regulation of PEPCK protein and phosphorylation of insulin receptor substrate-2.[30] The antihyperglycemic effect of UA in hyperglycemic rats was mediated by insulin secretion and insulinomimetic effect on glucose uptake and synthesis and translocation of GLUT4.[31]

Antifungal

In a study performed by Mahlo et al.,[32] UA with MIC of 20–250 mg/mL showed antifungal activity against *Aspergillus niger, Aspergillus parasiticus, Colletotrichum gloeosporioides, Trichoderma harzianum, Penicillium expansum, Penicillium janthinellum* and *Fusarium oxysporum*. At different concentrations (500, 750, and 1000 ppm), UA also significantly inhibited ($P < 0.001$) the spore germination of *Alternaria alternata, Cochliobolus lunata, Fusarium moniliforme, Fusarium pallidoroseum*, and *Helminthosporium*.[33]

Anti-viral effect

Zhao et al.[34] evaluated the anti-viral effect of UA against cytomegalovirus in comparison to that of two other drugs, ganciclovir and jinyebaidu (control). They concluded that the antiviral activity of UA is significantly stronger than that of ganciclovir or jinyebaidu. In another study, UA presented anti-HCV (hepatitis C virus) effect through the suppression of HCV NS5B RdRp activity by acting as a noncompetitive inhibitor.[35]

Trypanocidal effect

UA exhibited *in vitro* trypanocidal effect as shown by an IC50 value of 25.5 μM and 77% trypomastigote lysis at concentration at 128 μM. In the in vivo assay, the administration of UA at 20 mg/kg/day significantly reduced the parasitemia.[36]

3. Drug delivery system

UA exhibits various pharmacological effects. However, it has low solubility in aqueous medium (1.02×10^{-4} mg/L at 25° C) which affects its bioavailability and therapeutic application, because the solubility and polarity of a substance can influence its ability to penetrate biological membranes.[37,38] Various strategies can be employed to overcome these limitations, such as particle size reduction, salt formation, chemical modifications of molecules, use of surfactants, pH adjustment or incorporation of the drug into different delivery systems.[39] Drug delivery systems have been used with great success to improve the physicochemical properties of UA and favor its therapeutic application.

They also modulate drug release besides to improve solubility and increase the bioavailability of hydrophobic drugs.[40,41] Several nanocarriers have been used for UA delivery, such as nanoemulsions, mesoporous silica nanoparticles, solid lipid nanoparticles, polymeric nanoparticles, liposomes, niossomal gels, and solid dispersions.

In a study performed by Li *et al*,[9] pH-sensitive mesoporous silica nanoparticles were found to be biocompatible and allowed sustained release of UA, besides enhancing the cytotoxic effect against HepG2 human hepatocellular carcinoma cells, compared with free UA. Vargas de Oliveira *et al.*[8] evaluated trypanocidal effect, cytotoxicity and in vitro dissolution profile of UA delivered by the nanoemulsions (constituted by purified water, Capryol®90 and Cremophor® EL/Transcutol® P) and investigated the improvement in the drug dissolution profile. UA release was found to be 3.75 times higher and 24 times faster than that from a physical mixture in alkaline dissolution medium, which indicated the importance of emulsification in enhancing the availability of the drug in the dissolution medium.

Nahak et al.[10] investigated the physicochemical characteristics of the UA-loaded into solid lipid nanoparticles and found that the crystallinity of UA was lost due to its incorporation into the systems. In addition, the lipid matrix affected the rate of drug release and drug release pattern, exhibiting an antitumor effect greater than that of free UA against human leukemic cell line (K562) and melanoma cell line (B16). Long-circulating and pH sensitive liposomes containing UA (SpHL-UA) were used to improve the intravenous drug administration and its antitumoral effect on breast (MDA-MB-231) and prostate (LNCaP) cancer cell lines.[11] In a study performed by Zhang et al.,[42] UA loaded nanoparticles using amphiphilic mPEG–PCL block copolymers exhibited a sustained drug delivery profile and improve the antitumor effect of UA through the inhibition of COX-2 and activation of caspase-3 in gastric tumor cells.

Topical formulations were also used owing to its advantages over the oral route, such as the absence of gastrointestinal effects, reduced renal toxicity, and greater patient compliance.[43,44] Transdermal niossomal gels of UA developed for the treatment of arthritis resulted in a vesicle size of 665.45 nm, drug incorporation efficiency of 92.74%, and significantly superior in vivo compared with oral formulation and conventional gel system of UA.[12]

Solid dispersions can also be an interesting approach to the delivery of UA because these, comprise molecular mixtures of hydrophobic drugs with carriers that are hydrophilic or amphiphilic in nature, which in addition to allowing a reduction of drug particle size can improve the solubility and modulate drug release profile.[45] An experiment performed by Eloy and Marchetti[13] to evaluate the influence of hydrophilic carriers (PEG 6000 and Poloxamer 407) and the method of preparation (fusion and solvent) of solid dispersions for aqueous solubility of UA showed that Poloxamer 407 increased the solubility and dissolution rate of the drug in solid dispersions more efficiently than PEG 6000 because of its surfactant properties. In addition, the solvent method for obtaining solid dispersions was more efficient than the fusion method for increasing drug solubility.

4. Analytical methods

The development and validation of analytical methods are important in the discovery phases, development, and manufacture of pharmaceuticals. Analytical method validation guarantees that the performance characteristics of the method employed are suitable for its intended use.[46] With the introduction of new drug molecules, such as UA with no standards and analytical procedures in pharmacopeias, analytical method development and validation has become important.

Several analytical methods have been studied for qualitative and quantitative analyses of UA in various raw materials. Among most studied the analytical methods for the analysis of UA are HPLC,[47] coupled gas chromatography-mass spectrometry (GC–MS),[48] ultra-performance liquid chromatography (UPLC),[49] cyclodextrin-modified micellar electrokinetic

Table 1. HPLC methods reported in literature for analysis of the UA in plants.

Reference	Method	Matrix	Column	Mobile Phase	Linearity	F_R(mL/min)	R_t(min)
[64]	HPLC- PAD/UV	*Perilla frutescens*	Spherisob ODS RP-C18, (250 × 4.6 mm, 5 μm)	C_2H_3N:1.25% aqueous H3PO4 (86:14, v/v)	2.5 – 62.5 μg/mL	0.5	~ 19
[65]	HPLC- UV	*Eriobotrya japonica*	ODS RP-C18 (250 × 4.6 mm, 5 μm)	MeOH: 0.03 mol/L $PO4^{3-}$ buffer, pH 2.8 (88:12, v/v)	50 – 400 μg/mL	1.0	19.86
[66]	HPLC- DAD/UV	*Macrocarpium officinale*	Kromosil RP- C18 (250 × 4.6 mm, 5μm)	MeOH: H_3PO_4: H_2O (88:0.05:11.95,v/v/v)	1.01–6.06 μg/μL	1.0	20
[67]	HPLC- DAD/UV	*Oldenlandia diffusa*	Zorbax Eclipse XDB-C18 (250 × 4.6 mm, 5 μm)	MeOH: 0.2% aqueous ammonium acetate, pH 6.74 (83:17,v/v)	13.5 -541.6 μg/mL	1.0	—
[56]	HPLC- DAD/UV	*Prunellae Spica*	Phenomenex C18 (250 × 4.6 mm, 5 μm)	MeOH: 0.01 M $PO4^{3-}$buffer, pH 2.8 (88:12, v/v)	10 – 300 μg/mL	0.8	—
[55]	HPLC- DAD/UV	*Olea Europaea*	ODS Hypersil C18 (250 × 4.6 mm, 5 μm)	C_2H_3N: 0.02 M $PO4^{3-}$buffer, pH 3.5 (55: 45, v/v) with 7.5 mM HP-γ-CD	25–300 μg/mL	1.0	30
[68]	HPLC- UV	*Ziziphora clinopodioide*	Kromasil C18 (150 × 4.6 mm, 10 μm)	MeOH: 0.03 M $PO4^3$ buffer, pH 3,0 (90:10 v/v)	0.6 – 1.8 mg/mL	0.5	22.79
[69]	HPLC- FLD	*Crataegus pinnatifida*	Hypersil BDS C 8 (200 × 4.6 mm, 5 μm)	A: C_2H_3N: H_2O (30:70, v/v); B: 100% C_2H_3N	0.05- 6.5 μg/mL	1.0	—
[70]	HPLC- UV	*Vitex Negundo*	Symmetry® RP-C18 (250 × 4.6 mm, 5 μm)	C_2H_3N: MeOH (80:20, v/v)	0.01–0.1 mg/mL	0.5	12.36
[71]	HPLC- UV	*E. tereticornis.*	Symmetry® RP- C18 (250 mm x 4.6, 5 μm)	MeOH: H_2O acidifed with TFA, pH 3.5 (88:12, v/v)	0.05–0.3 mg/mL	1.0	9.7
[72]	HPLC- UV	*Eriobotrya japonica*	Alltech®Apollo™ RP-C18 (250 mm x 4.6, 5 μm)	MeOH: H_2O (95:5, v/v)	25–300 μg/mL	0.4	21.57
[73]	HPLC- PAD/UV	*Lamii albi flos*	LiChrospher®100 RP-C18 (250 × 4 mm, 5 μm)	C_2H_3N: H_2O: 1% aqueous H_3PO_4 (85:15:0.5, v/v/v). IS.	5–100 μg/mL	0.8	—
[47]	HPLC-UV	*Plumeria obtusa*	Kromasil 100 C18				
(250 × 4.6 mm, 5 μm)	C_2H_3N: H_2O (85:15, v/v). IS.	3.62–116 μg/mL	1.0	15			
[49]	HPLC-MS	*Olea europaea*	Zorbax Eclipse PAH C18 (150 × 4.6 mm, 3.5 μm)	MeOH: H_2O (83:17). IS.	0.005–15 μM	0.8	—
[74]	HPLC-MS	*S. racemosa, C. impatiens, M. henrici, D. alum tanguticum Maxim, C.pulmonarium, P. granatum*	Hypersil C18 (200 × 4.6 mm, 5 μm)	A: (H_2O + 5% C_2H_3N) B: (100% C_2H_3N). GR.	4 – 2000 ng/mL	1.0	5

HPLC: High performance liquid chromatography. RP: reversed-phase. Rt: retention times. F_R: flow rate. UV: ultraviolet detector. DAD: diode-array detector. PAD: photodiode array detector. FLD: fluorescence detector. MeOH: methanol. C_2H_3N: acetonitrile. H_3PO_4: phosphoric acid. $PO4^{3-}$ buffer: phosphate buffer. TFA: trifluoroacetic acid.

Table 2. HPLC methods reported in literature for analysis of the UA in biological fluids.

Reference	Method	Matrix	Column	Mobile Phase	Linearity	F_R(mL/min)	R_t(min)
[75]	HPLC MS	Rat plasma	Hypersil™ C18 (250 × 4.6 mm, 5 μm)	MeOH: H_2O (95:5, v/v).	10–1000 ng/mL	1.0	2.3
[76]	HPLC MS	Rat plasma	Sun Fire RP-C18 (150 × 10 mm, 10 μm)	A: C_2H_3N:H_2O (30:70, v/v); B: 100% C_2H_3N).	5–200 ng/mL	2.0	24
[54]	HPLC MS	Rat plasma	Chromasil C18 (250 × 4.6 mm, 5 μm)	MeOH: 0.1% aqueous formic acid (86:14, v/v).	0.94–462 ng/mL	1.0	13
[77]	HPLC UV	Rat plasma	RP- C18 (250 × 4.6 mm, 5 μm)	C_2H_3N: 0.5% triethylamine, pH 4.0 (70:30, v/v).	25–150 ng/mL	1.0	—

HPLC: High performance liquid chromatography. RP: reversed-phase. Rt: retention times. F_R: flow rate. MS: Mass spectrometry. MeOH: methanol. C_2H_3N: acetonitrile.

chromatography (CD-MEKC),[50] micellar electrokinetic capillary chromatography (MECC),[51] nonaqueous capillary electrophoresis (NACE),[52] high performance thin layer chromatography (HPTLC);[53] MS,[54] and nuclear magnetic resonance (NMR).[55]

Most of the analytical methods reported in the literature for the analysis of UA in different matrixes use the HPLC technique, possibly due of its simplicity, excellent sensitivity, resolution, and short analysis time.[56] In addition, it is a widely used technique in the pharmaceutical industry for monitoring, evaluating and quality control of finished products despite the high operating cost.[57]

Most studies have reported UA separation by HPLC using C18 analytical columns and methanol-water or acetonitrile-water as the mobile phase with the pH adjusted between 2.8 and 6.74 using various organic additives (phosphate buffer, phosphoric acid, ammonium formate, formic acid, orthophosporic acid, and trifluoroacetic acid). The separation was conducted more frequently with isocratic elution than gradient elution. Retention times ranged from 2.3 to 30 min with a flow rate of 0.4–2 mL. min^{-1}. The HPLC methods reported in the literature for the analysis of UA in plants, biological fluids, and drug delivery systems have been presented in Tables 1, 2, and 3.

Among coupled HPLC detectors used for UA analysis, the ultraviolet detector was the most common (200–215 nm). Diode array detector (DAD) and photodiode array detector (PAD) were also used as well as fluorometry (FLD) and MS. However, the accurate detection of this compound using absorptiometry has several challenges because UA lack chromophores, and exhibits low UV and fluorescence absorption. Furthermore, there are matrix interferences, such as in the case of plant samples, which have several substances with similar structures and polarities.[55,58]

Li *et al*[59] reported an approach to increase the selectivity and sensitivity of UA and OA detection in medicinal herbs using a new dual-sensitive probe, 2-(5-benzoacridine)ethyl ptoluenesulfonate (BAETS), as a pre-column labeling reagent coupled with the HPLC-FLD method. Furthermore, an HPLC-MS method developed to analyze pentacyclic triterpenes in *Olea europaea L* showed improved sensitivity with a limit of quantification of 2 nM for UA.[60]

Other hand, the other analytical methods were also used successfully to evaluate UA. An HPTLC method for the identification of UA and OA in the methanol extract of *W. volubilis* was investigated by Gopal et al.[61] using a mixture of petroleum ether: chloroform: ethyl acetate: methanol (4:1:0.1:0.1) as mobile phase for UA. The method showed linearity at 10–25 μg/mL and confirmed the presence of OA and UA in the methanol extract. Patel and Vyas[62] studied the stability of UA under stress conditions (acid hydrolysis, alkaline hydrolysis, oxidation, photolysis, dry heat and humidity) and developed a stability indicating HPTLC method that enables separation of the drug from its main degradation products. The authors used pre-coated silica gel TLC plates and toluene: ethyl acetate: formic acid (7:3:0.1, v/v/v) as the mobile phase. Densitometric detection and quantification (530 nm) showed linear relationship at 200–600 ng/spot.

Caligiani et al.[48] developed a GC–MS method for the determination of UA in commercial plant extracts. This technique was chosen due to its high selectivity and precision in the analysis of complex matrices. The method exhibited linearity in the 20–5000 mg/kg range, a limit of quantitation of 10 mg/kg (S/N ratio 10), and a limit of detection of 3 mg/kg (S/N ratio 3.3), indicating good flexibility in the analysis of several types of extracts.

A two-dimensional NMR method was compared with an HPLC method for the quantification OA and UA in plant extracts of the *Lamiaceae* and *Oleaceae* family.[55] The current study showed that the use of proton–carbon heteronuclear single-quantum coherence (1H–13C HSQC) in combination with the proton–carbon heteronuclear multiple-bond correlation (1H–13C HMBC) NMR spectroscopy enables the bond of the proton and carbon-13 spins across the molecular backbone

Table 3. HPLC methods reported in literature for analysis of the UA in drug delivery systems.

Reference	Method	Matrix	Column	Mobile Phase	Linearity	F_R(mL/min)	R_t(min)
[78]	HPLC UV	Polymeric nanoparticles	RP- C18 (250 × 4 mm; 5 μm)	MeOH: 0.1% aqueous H_3PO_4 (80:12, v/v).	10–310 μg/mL	1.0	—
[79]	HPLC UV	Solid dispersions	Lichrospher® RP C18 (250 × 4 mm; 5 μm)	C_2H_3N: H_2O (88:12, v/v).	1.0–50.0 μg/mL	1.0	11
[11]	HPLC UV	Liposomes	LiChrosorb RP-C18 (250 × 4 mm, 5 μm)	MeOH: H_2O (1 : 1, v/v).	5–60 μg/mL	1.5	—
[80]	HPLC MS	Nanocrystals	Zorbax SB C18 (4.6 × 250mm, 5 μm)	C_2H_3N: 0.1% aqueous H_3PO_4 (85:15,*v/v*)	7.8–2.0 ng/mL	1.0	—

HPLC: High performance liquid chromatography. RP: reversed-phase. Rt: retention times. F_R: flow rate. UV: ultraviolet detector. MS: Mass spectrometry. MeOH: methanol. C_2H_3N: acetonitrile. H_3PO_4: phosphoric acid.

promoting the identification these triterpenoids acid without the need for physicochemical separation and, thus allowing a rapid quantitative analysis (~14 min) and in accordance with the data obtained by HPLC.

Wang[63] investigated a near-infrared spectroscopy (NIRS) method for the quantitative analysis of OA and UA in *Fructus ligustri lucidi*. The proposed method was efficient as a comparative HPLC method as the values of the t-tests for NIRS and HPLC method were 0.590 and 0.441, respectively both less than t(0.05,19) ¼ 2.093 with a 95% confidence level. Therefore, the results of the validation sets obtained by HPLC and NIRS presented no significant statistical difference.

Liu[51] studied an MECC method to determine UA and its isomer in *Ligustrum lucidum* using a buffer (15 mmol/L disodium hydrogen phosphate, 15 mmol/L disodium tetreborate, 10 mmol/L SDS and 5% v/v alcohol) UV detection at 214 nm at an applied voltage of 16 kV. An MEKC method developed by Yang[50] for the determination and quantification of OA and UA in *Pterocephalus hookeri* using a buffer (50 mM borax, 10% ethanol (v/v) and β – cyclodextrin at 8 mM, pH 9.53) with an application voltage of 20 kV and detection at 214 nm resulted in a good separation of OA and UA.

Nonaqueous capillary electrophoresis involves the use of electrolyte solutions obtained from pure organic solvents and allows the solubility and stability of hydrophobic substances.[52] In a study performed by Qi et al.,[52] the NACE method was demonstrated to be suitable for simultaneous separation the UA and OA using a methanol:acetonitrile (65:35,v/v) mixture containing 90 mm trishydroxymethylaminomethane (Tris) at an applied voltage of +25 kV and, UV detection at 214 nm.

5. Conclusion

According to the studies presented in this review we can conclude that UA exhibits several biological effects, which indicate its potential in the treatment and prevention of various pathologies. In addition, different delivery systems for UA have been developed, such as nanoparticles and solid dispersions, which improve the biopharmaceutical properties of this molecule, favoring its therapeutic application.

The majority of presently applied methods for determining UA in plants use HPLC. With this review, we could verify that new methods are being introduced for UA analysis, which use other effective and accurate techniques as NMR. Furthermore, although most studies have reported the use of plants matrix for the development and validation of analytical methods for UA by HPLC, a few studies have evaluated this compound in biological fluids and drug delivery systems.

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