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Analytical Methods for Characterizing Bioactive Terpene Lactones in *Ginkgo Biloba* Extracts and Performing Pharmacokinetic Studies in Animal and Human

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1. Introduction

Ginkgo biloba is an ancient Chinese tree, appeared more than 250 million years ago, and the only surviving member of Ginkgoaceae family [Schmid, 1997]. *Ginkgo biloba* was used as herbal remedy for many centuries in China, and now its extracts are one of the most widely used herbal products in the world, especially in the United States and in Europe [Blumenthal, 2000; Mahadevan & Park, 2008]. *Ginkgo biloba* extract is considered an alternative medicine for the treatment and/or the prevention of different pathologies and in some cases it could be suggested to be used as complementary of the mainstream medicine [Ernst, 2000]. In fact, over the past decades, there was a steady growth trend in the use of these alternative treatments. In particular, concentrated and partially purified products, containing *Ginkgo biloba* active constituents, have been marketed widely in the world for the treatment of cognitive deficits and other age-associated impairments [Kanowski et al., 1996; Le Bars et al., 1997]. Furthermore, it has been used as therapeutic compound for many other chronic and acute forms of diseases such as cardiovascular and bronchial pathologies [Diamond et al., 2000].

In view of the large market as well as the keen interest in the use and rediscovery of these herbal products throughout the world, the quality control of *Ginkgo biloba* extracts becomes necessary, in order to guarantee their clinical efficacy and safety. Therefore, it is important to monitor simultaneously the bioactive constituents present in *Ginkgo biloba* extracts, optimizing the analysis time and reducing costs. In fact, in the recent years, numerous groups reported in literature different analytical methods, using various chromatographic conditions and spectrophotometric technologies, to create quick, accurate and applicable analytical approaches for the identification and the chemical structure characterization of *Ginkgo biloba* constituents.

Ginkgo biloba extracts contain a large number of representative constituents such as terpenoids, polyphenols, allyl phenol, organic acids, carbohydrates, fatty acids and lipids, inorganic salts and amino acids. However, the pharmacological activity of *Ginkgo biloba*

extracts was attributed to the synergistic action of two distinct classes of chemical compounds, the flavonoids and the terpene trilactones [Sticher, 1993; Stiker et al., 2000; Li & Fitzloff, 2002a; Van Beek, 2002; Smith & Luo, 2004]. The flavonoids comprise a large group of polyphenols and include flavone and flavonol glycosides, acylated flavonol glycosides, biflavonoids, flavan-3-ols and proanthocyanidins. Of these, flavonol glycosides are more abundant than the other ones. Moreover, numerous flavonol glycosides were identified in *Ginkgo biloba* extracts as derivatives of the aglycones such as quercetin, kaempferol and isorhamnetin that are usually present in the leaves in relatively small amounts [Haslet et al., 1992; Van Beek, 2002]. The flavonoids are known to act mainly as antioxidants [Goh et al., 2003], free radical scavengers [Ellnain-Wojtaszek et al., 2003] and cation chelators [Gohil & Packer, 2002]. Finally, they could play a protective role in the prevention of certain kind of cancer as suggested in different studies on animal models [Kuo, 1997; Kandaswami et al., 2005].

The second group is represented by the terpene trilactones, which include diterpenoid (ginkgolides) and a sesquiterpenoid (bilobalide) compounds. Ginkgolides A, B, C, J, K, L and M are potent and selective antagonist of platelet activating factor (PAF) [Braquet, 1987; Van Beek et al., 1991; Smith et al., 1996; Hu et al., 1999]. PAF is an endogenous and highly active mediator of inflammation in the human body; it is produced by a variety of inflammatory cells and for this reason it is implicated in various disease states. So, the ginkgolides, used as the PAF antagonist, are able to prevent and treat thrombosis, illness of blood vessel of heart and brain, arrhythmia, asthma, bronchitis and allergic reactions [Chavez & Chavez, 1998; Sticher, 1999; Diamond et al., 2000; Koch, 2005].

On the other hand, the sesquiterpene bilobalide exhibits neuroprotective properties [Chandrasekaran et al., 2001; Defeudis, 2002]. It is widely employed to treat symptoms associated with mild-to-moderate dementia, impairment of other cognitive functions associated with ageing and senility and related neurosensory problems [Blumental et al., 2000]. In fact, numerous studies, based on in vivo models, indicated that the administration of bilobalide can reduce cerebral edema due to triethyltin, decrease cortical infarct volume as verified in certain stroke models and reduce damage caused by cerebral ischemia [Chandrasekaran et al., 2001; Defeudis, 2002].

All the mentioned pharmacological actions of the compounds isolated from *Ginkgo biloba* were clarified over the years and helped to highlight the diversity of their potential activities on human health. In particular, in the present chapter we focused our attention to review the neuroprotective role of *Ginkgo biloba* extracts. In fact some publications, reporting the pharmacokinetic behaviours and in vitro and in vivo clinical results of *Ginkgo biloba* extracts, shown that they are an important ingredient to treat cognitive disturbance, although the molecular mechanism of their action is still ambiguous. In particular we examined bilobalide, the bioactive compound of *Ginkgo biloba* that is probable the principal responsible for this effect.

Finally, this chapter aims to provide an overview on the main techniques and methods used for the assay of *Ginkgo biloba* components.

2. Neuroprotective properties of *Ginkgo biloba* extracts and Bilobalide

Ginkgo biloba extract (GBE) presents a wide range of biological/therapeutical effects. Concerning its pharmacological activity on the central nervous system it seems due to synergic action of its main constituents: flavonoid-glycosides and terpene-lactones.

The investigations of neuroprotective effects of *Ginkgo biloba* have used its standardized extract. The extract standardized contains about 24% flavonoid glycosides and 6% terpene lactone.

Specifically *Ginkgo biloba* extract (GBE) is described to have different biological effects. For example, several authors reported that GBE may be a molecular target of amyloid precursor protein (APP) [Luo et al., 2002; Agustin et al., 2009; Jin et al., 2009] and it determined beneficial effects on brain function.

Other authors investigated the action of GBE on oxidative damage [Bridi et al., 2001; Naik et al., 2006; Sener et al., 2007], specifically in relation to ischemia/reperfusion [Urikova et al., 2006; Domorakova et al., 2009]. In addition, it is reported that GBE protects against mitochondrial dysfunction in platelets and hippocampi [Shi et al., 2010a; Shi et al., 2010b].

Ginkgo biloba extract it was also reported to have a positive effect on memory in healthy animals [Gong et al., 2006; Yamamoto et al., 2007; Blecharz-Klin et al., 2009] and humans [Kennedy et al., 2007].

Of course a number of authors concern the effect of GBE on typical neuro-degeneration diseases, such as Alzheimer [Agustin et al., 2009; Luo, 2006; Ahlemeyer & Krieglstein, 2003; Luo, 2001] and Parkinson [Beal, 2003; Kim et al., 2004; Ahmad et al., 2005; Chen et al., 2007; Rojas et al., 2008].

Regarding the flavonoid fraction of GBE only few studies have been performed and they concern prevention of membrane damage caused by free radicals. In particular, flavonoid fraction protects cultures of neurons against oxidative stress due to hydrogen peroxide and iron sulfate [Sloley et al., 2000], as well as neural tissue against cerebral ischemia lesion [Dajas et al., 2003]. Moreover, it was described that flavonoid fraction inhibited sodium nitroprusside-induced death in primary hippocampal cultures of rat [Saija et al., 1995], and the authors suggested that flavone glycosides, as radical scavengers, block the formation of peroxynitrite as a product of NO and superoxide anion reaction.

Concerning the terpene-lactones, studies mainly regard bilobalide. In vitro and ex vivo investigations indicate that bilobalide has multiple actions, such as preservation of mitochondrial ATP synthesis [Janssens et al., 1995], inhibition of apoptotic damage [Ahlemeyer et al., 1999], suppression of hypoxia-induced membrane deterioration [Klein et al., 1997] and increasing the expression of the mitochondrial DNA-encoded COX III [Chandrasekaran et al., 2001].

Specifically, the sesquiterpene reduces the edema formation in hippocampal slices exposed to N-methyl-D-aspartate (NMDA) [Kiewert et al., 2007], or obtained by oxygen-glucose deprivation (OGD) [Mdzinarishvili et al., 2007]. The neuroprotective effect of bilobalide is partially correlated to its GABAergic antagonism, but it doesn't fully explain the bilobalide's action [Kiewert et al., 2007]. More recently, it has been reported that glycine, at 10-100 mM level, contrasts the effect of bilobalide. In particular, bilobalide reduces the release of glycine during ischemia but it does not interact with glycine receptors [Kiewert et al., 2008].

Because bilobalide is instable, it has been prepared a stable derivative called NV-31. This modified compound resulted to reduce by 50% the cellular ROS content in chick neurons submitted to serum deprivation and staurosporine-induced apoptosis [Ahlemeyer et al., 2001]. Moreover NV-31 has been reported to potentiate hippocampal neuron recombinant glycine receptor Cl channels [Lynch & Chen, 2008].

The protective effect of bilobalide against convulsion was observed by Sasaki et al. (2000) using 4-O-methylpyridoxine (MPN) for changing the levels of gamma-aminobutyric acid (GABA) and glutamic acid decarboxylase (GAD) activity in hippocampus cerebral cortex.

Finally, ginkgolide B (GB) was used in neuroprotective studies. In particular, this terpenolactone reduced up-regulation of constitutive and inducible nitric oxide synthase in hyperthermic brain injury [Sharma et al., 2000].

3. Structural characterization of *Ginkgo biloba's* main compounds

Ginkgo biloba is characterized by the presence of numerous constituents belonging to different chemical classes, which are well investigated over the years. In fact, there are many studies that report the various groups of components present in its extracts [Van Beek, 2002; Singh et al., 2008; Van Beek & Montoro, 2009]. However, depending on their chemical structures, the major bioactive compounds can be classified into two groups: flavonoids and terpene lactones [Li & Fitzloff, 2002a; Li & Fitzloff, 2002b].

The flavonoids, also called phenylbenzopyrines or phenylchromones, comprise a large group of structurally related compounds, characterized by the presence of two aromatic rings and a heterocyclic ring with one oxygen atom; this group include flavonol glycosides, biflavonoids, biflavones, proanthocyanidins and isoflavonoids. Specifically, the flavonoids most commonly present in *Ginkgo biloba* extracts are the flavonol glycosides, in which one or more hydroxyl group of the aglycones are bound to a carbohydrate moiety, usually via the 3 or 7 position (Fig.1). Numerous flavonol glycosides were identified as derivatives of the phenolic aglycones (quercetin, kaempferol or isorhamnetin) that, when alone, are present in relatively low concentration [Hasler et al., 1992; Sticher, 1999]. However, the *Ginkgo biloba* contains also a large number of biflavonoids, which are flavonoid-flavonoid dimers connected by a C–O–C or C–C bond.

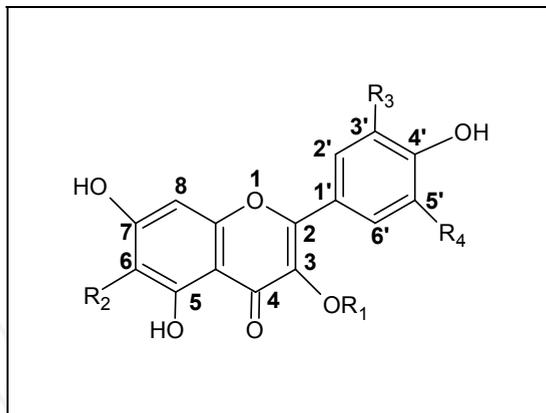


Fig. 1. Structural skeleton of flavonoids.

Terpene lactones include 20-carbon diterpene lactone derivatives (ginkgolides) and a 15-carbon sesquiterpene (bilobalide). These compounds are the unique natural products to possess a tert-butyl group in their structure [Van Beek, 2005] (Fig.2). In particular, ginkgolides contain a rigid carbon skeleton consisting of six fused 5-membered carbocyclic rings, that is, a spiro [4.4] nonane carbocyclin ring, three lactones and a tetrahydrofuran. On the contrary bilobalide has a more flexible structure containing only 5-membered rings [Nakanish et al., 1971].

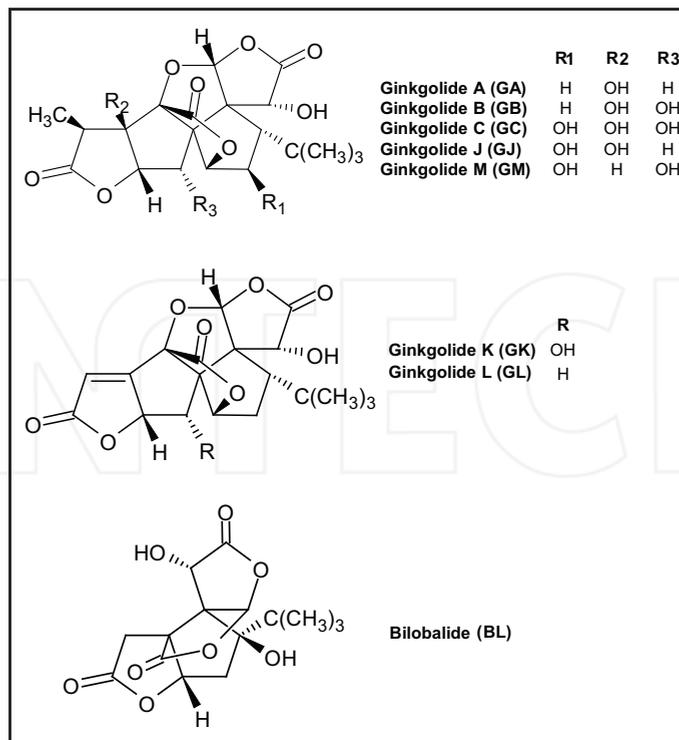


Fig. 2. Chemical structures of the terpene trilactones of *Ginkgo biloba* extract.

By far the terpene lactones received a great attention for the chemical uniqueness, due to their cage like structure. Ginkgolide A, B, C and M were isolated for the first time from *Ginkgo biloba* root bark and described by Furukawa in 1932 (1932), and only later, ginkgolides A, B and C were reported to be present in the leaves too. Ginkgolide J was identified, by Weings et al. in 1987 (1987), as a minor constituent present in the leaves of *Ginkgo biloba*. In addition, Wang et al. (2001) reported the identification of other two ginkgolides (K and L) containing a further double bond. In fact, Yuan et al. (2008) recently described ginkgolide K as the dehydrated form of ginkgolide B. Similarly, ginkgolide L should derive from the dehydration of ginkgolide A, although this hypothesis is not confirmed in literature.

A thorough mass spectrometric investigation of this class of compounds is very important for their identification and characterization.

The fragmentation pathway of bilobalide observed in our laboratory is shown in Fig.3. It was based on data obtained by means of LC-MS/MS analysis with an APCI source and an ion trap analyzer (ITMS), in negative ion mode. These results are in good agreement with those observed by Sun et al. (2005) using an electrospray interface. Instead, the fragmentation of bilobalide obtained by LC-ESI-MS/MS using a triple quadrupole (QqQ) analyzer, shows differences related to the relative abundances of the fragmented ions. Specifically, the most abundant fragments are m/z 163 and 251 from QqQ and ITMS, respectively.

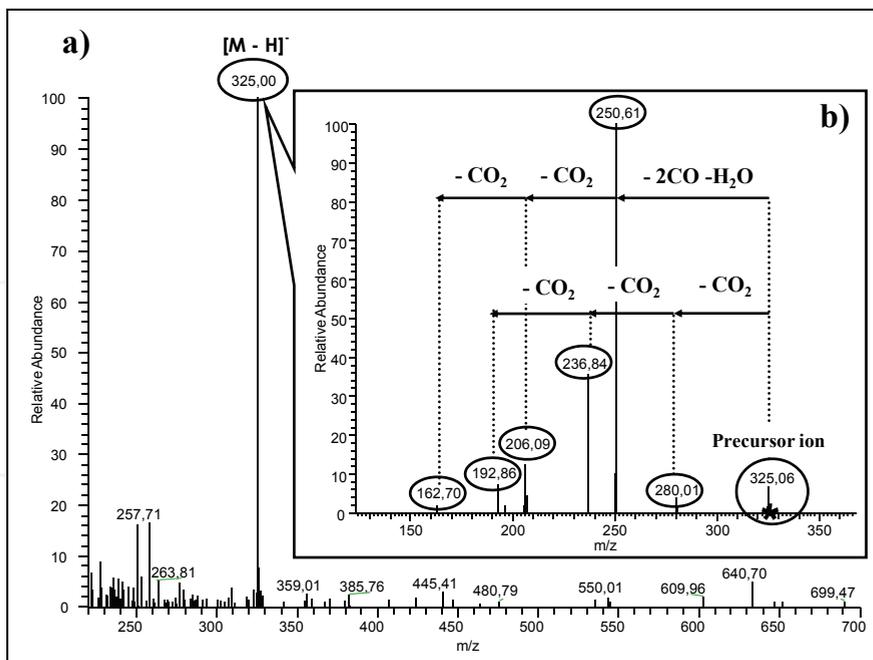


Fig. 3. a) APCI-MS and b) APCI-MS/MS spectra of $[M-H]^-$ at 325 m/z .

Table 1 reports the fragmented bilobalide ions obtained by ion trap and triple quadrupole. In particular, ion product at m/z 325 is due to the loss of a ter-butyl and a hydroxyl group, while fragmentation at m/z 163 is related to the loss of two carbon dioxide molecules.

Ion	Bilobalide m/z	% relative abundance	
		HPLC/ESI-MS/MS [Sun et al.; 2005]	HPLC/APCI-MS/MS
$[M-H]^-$	325	30	6
$[M-H-CO_2]^-$	281	0	4
$[M-H-2CO_2]^-$	237	25	36
$[M-H-3CO_2]^-$	193	30	8
$[M-H-2CO-H_2O]^-$	251	30	100
$[M-H-CO-H_2O-2CO_2]^-$	206	10	14
$[M-H-2CO-H_2O-2CO_2]^-$	163	100	2

Table 1. Comparison of the major product ions of the bilobalide obtained by using ESI-MS/MS and APCI-MS/MS methods.

On the other hand, the ginkgolides show fragmentation pathways similar among them. Generally, the most favourable fragmentation way of the deprotonated ginkgolides is the loss of single and multiple carbon monoxide molecules. In each cases, the most abundant fragment ion derived from the loss of two carbon monoxide molecule, $[M-H-2CO]^-$. For

example the MS/MS spectrum obtained from the ginkgolide A molecule ion $[M-H]^-$ 407 m/z gives a prominent ion product at m/z 351, resulting from the loss of two carbon dioxide molecules and a three less intense product ions at m/z 379, 363 and 319 due to the loss of one carbon monoxide molecule, one and two molecules of carbon dioxide, respectively [Van Beek, 2005; Sun et al., 2005].

4. Analytical methods for quality control of *Ginkgo biloba* extracts

Chemical fingerprint analysis represents a comprehensive approach for the quality assessment purpose of traditional Chinese herbs. In fact, most herbal medicines are complex mixtures whose therapeutic effect is often attributed to the cumulative effects of many components. For this reason, it is important to achieve an overall view of all the components present in the extracts to evaluate the quality of the plant products. Moreover, it is necessary and important to develop a reliable and applicable quality control method for the constituents present in most herbal extracts. To this end, different analytical methods are proposed for the quality and the stability evaluation of herbal medicines.

As described above, for the analysis of the flavonoids and terpene lactones, the main bioactive compounds present in *Ginkgo biloba* extracts, the scientific literature report a lot of methods. The great number of these methods is based on high-performance liquid chromatography (HPLC) coupled to UV [Pietta et al., 1990; Pietta et al., 1992; Hasler, et al., 1992], refractive index (RI) [Van Beek et al., 1991; Chen et al., 1998; Wang & Ju, 2000], or ELSD [Li & Fitzloff, 2002] detection and gas chromatography (GC) combined to flame ionisation (FID) [Huch & Staba, 1993; Van Beek, 2002; Yang et al., 2002] or mass spectrometry (MS) [Chauret et al., 1991; Deng & Zito, 2003] detection.

High-performance liquid chromatography coupled to the ultra-violet detection (HPLC-UV) is the technique of choice for the fingerprinting analysis of the total flavonoids of *Ginkgo biloba* L. extracts [Hasler et al., 1992], while it presents several limitations for the qualitative and quantitative determination of terpenes, due to their low UV absorption and the impurities present in the complex matrix of *Ginkgo biloba* L. extracts. However, Pietta et al. (1990; 1992) described a new procedure for the preparation of samples that, using HPLC combined to UV detector, permits the identification of terpene compounds of *Ginkgo biloba* L. extracts. In particular, in the study proposed by Pietta et al. in 1990 was developed an efficient method for the purification of terpene compounds in *Ginkgo biloba* L. extracts based on the separation of the ginkgolides fractions by means of prepacked alumina columns. The resulting cleaned samples were separated by isocratic elution on Microsorb C18 columns (analytical: 100 × 4.6 mm i.d., 3µm; Rainin Instrument Co., Woburn, MA, USA - semi-preparative: 250 × 10 mm i.d., 5 µm; Biolab Instrument) using 10% isopropanol and analyzed with an UV photodiode detector. These results show that, combining a new sample purification process together with the optimization of the chromatographic conditions, it is possible to obtain a simple method suitable for the determination of bilobalide, ginkgolide A, B and C in *Ginkgo biloba* L. extracts.

Several investigators instead applied refractive index (RI) as an alternative detection method [Van Beek et al., 1991; Chen et al., 1998; Wang & Ju, 2000]. As an example, Wang and Ju (2000) developed a rapid analytical method that, using HPLC on a C18 column with methanol-water-orthophosphoric acid as eluents combined to refractive index (RI) detector, permits the quantification of terpene lactones (bilobalide and ginkgolide A, B, C, J) in *Ginkgo biloba* L. extracts in only 20 min of analysis time. This method resulted to be more suitable

and was employed with considerable success, although the sensitivity and the baseline stability still remain a problem.

On the contrary, evaporative light scattering detection (ELSD) seems to solve the problems related to the baseline stability and permits to reach higher sensitivity, requiring small solvent consumption. So, even if it is a non-selective detector, this technique has gained in popularity over the last decades, due to its capacity to detect the number and size of non-volatile compounds. In fact, several papers reported the application of HPLC with ELSD detector for the routine determination of ginkgolides and bilobalide [Li & Fitzloff, 2002b; Tang et al., 2003; Dubber & Kanfer, 2006]. As an example, Tang et al. (2003), applied RP-HPLC-ELSD method for the quantitation analysis of terpene lactones in *Ginkgo biloba* extracts. These authors by means of a Dinamic C18 column, using methanol and water under isocratic conditions as mobile phase (33:67, v/v), obtained the separation of five terpene lactones in 40 min of analysis time (bilobalide and ginkgolide A, B, C, J). This method represented a big advantage in terms of selectivity and precision; however the narrow linearity intervals (from 100 to 800 µg/ml) produced through ELSD response represent the major inconvenience of such kind of detectors.

Another excellent separation technique for terpene lactones is represented by gas-chromatography coupled to flame ionization detection (GC-FID) [Huch & Staba, 1993; Lang & Wai, 1999; Van Beek, 2002; Yang et al., 2002] which is very reliable for the sensitivity and reproducibility. However, the sensitivity and selectivity of this separative technique could be further increased by the coupling with mass spectrometer detectors (MS) [Chauret et al., 1991; Biber & Koch, 1999; Deng & Zito, 2003]. Nevertheless GC-based methods require complicated and time-consuming sample preparation steps and compound derivatization.

In the recent years, these problems are solved with the development of several mass spectrometry instruments and taking advantage of the combination of these ones with HPLC separation systems. In fact, in literature are reported many works in which different MS techniques were coupled to HPLC for analysing *Ginkgo biloba* extracts.

In particular, different investigators developed HPLC methods combined to MS detection, using a thermospray interface (TSP) (Pietta et al., 1994; Caponovo et al., 1995). Briefly, Pietta et al. (1994), applied high-performance liquid chromatography interfaced with a thermospray ion source mass spectrometer (LC-TSP/MS) for the identification of various flavonol glycosides from *Ginkgo biloba*. While, Caponovo et al. in 1995, employed LC-TSP/MS analytical techniques for the rapid detection of ginkgo terpene lactones. LC-TSP/MS method is not very precise due to variability and poor stability of the TSP interface. On the contrary, the discovery of the API (atmospheric pressure ionization) interfaces and in particular of electrospray ionisation sources (ESI) allowed a soft ionization and a stable combination between HPLC separation and MS detection. As reported by Mauri et al. (1999), the method based on liquid chromatography coupled with electrospray mass spectrometry (LC-ESI-MS) is specific, reproducible, rapid and permits quantitative analyses of terpenoids in *Ginkgo biloba* extracts. In particular LC-ESI-MS method permitted the monitoring of terpene lactones in *Ginkgo biloba* extracts by means of quadrupole instrument (positive mode) coupled to a C18 columns and 20 min of analysis time in isocratic separation [Mauri et al., 1999].

Similar results were obtained by Jensen et al. (2002) using a triple quadrupole equipped with an atmospheric pressure chemical ionization (APCI) interface in the negative ion mode and a methanol gradient. For increasing the sensitivity quadrupole needs selectively detection of single ion monitoring (SIM).

However, in this way, increasing the number of selected ions monitored, the sensitivity decreases and it is not possible to monitor unexpected ions. These problems were solved with the introduction of ion trap mass spectrometer (ITMS). In fact ITMS present the same sensitivity in full scan and SIM modes. Ding et al. (2008a) characterized the flavonoid and terpene compounds of *Ginkgo biloba* products by using a C18 capillary column coupled to ion trap MS. The use of the negative ion mode combined to the data depending scan for MS/MS acquisition lead to the characterization of more than 70 components from the *Ginkgo biloba* in 140 min of analysis time. As another example Chen et al. (2005) used a 28 min HPLC separation, based on a C18 analytical column, coupled to sonic spray ionization source (SSI) and a mass spectrometer equipped with a ion trap analyzer for characterizing terpene lactones constituents from *Ginkgo biloba* extracts in positive ion mode. On the other hand, Mauri et al. (2001) used liquid chromatography/atmospheric pressure chemical ionization for coupling HPLC with ion trap mass spectrometry (LC/APCI-IT-MS) to study the pharmacokinetics of terpene lactones in human. In particular, in this study chromatographic separation was achieved in less than 8 min and calibration curve was linear over a concentration range of 5-2000 ng/ml.

In addition to the ion trap analyzer, Ding et al. (2008b) employed a quadrupole time-of-flight (QTOF) mass spectrometer for characterizing terpene lactones (bilobalide and ginkgolide A, B, C,) in *Ginkgo biloba* L. extracts [Ding et al., 2008b]. Specifically, the authors used both analyzers to obtain two specific goals: ion trap MS for the characterization of the terpene fragmentation pathways and QTOF for the estimation of fragment ion mass accuracy (3-5 ppm) and the confirmation of the structural identification.

More recently, in our laboratory, the accurate mass measurement of the *Ginkgo biloba* terpene lactones was performed by means of the Exactive (Thermo Electron Corporation, San José, CA, USA) a non-hybrid mass spectrometer based on the Orbitrap technology. This instrument, equipped with a nanoelectrospray ion source, allows the acquisition of accurate mass data together with the improvement of sensitivity.

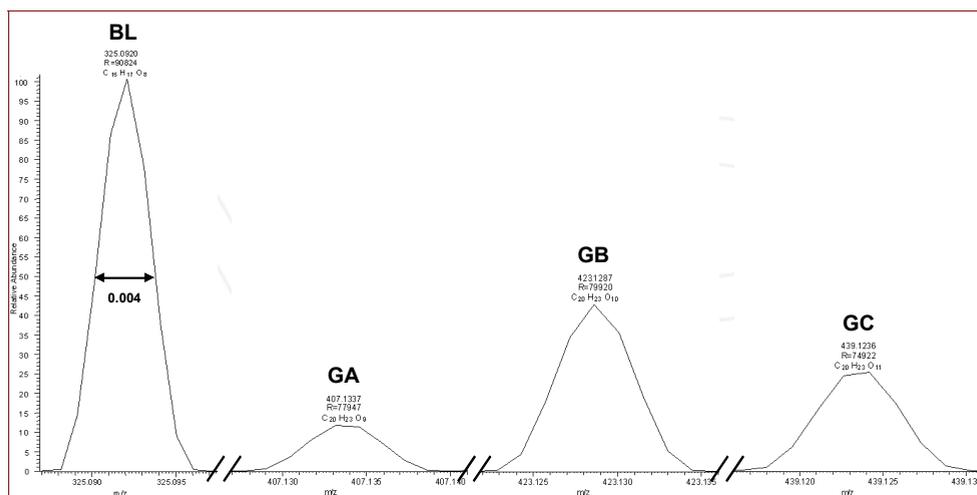


Fig. 4. MS spectra zoom of $[M-H]^-$ ions of bilobalide and ginkgolides A,B,C.

Fig. 4 reports the MS spectrum of [M-H]⁻ ions of bilobalide and ginkgolides A,B,C. The full scan spectra were acquired at a resolution setting of 100,000 in an infusion experiment calibration (i.e. no lock masses were used) and a high dynamic range. In particular, for all the terpene lactone ions showed in Fig. 4 it was possible to achieve narrow peak widths with a mass accuracy and a resolving power of around 0,2 ppm and 8×10^5 , respectively.

High resolution, accurate mass measurement together with high dynamic range are required for unequivocal characterization of mixtures even in the absence of precursor ion mass selection. However, if it is needed, additional informations can be provided by high resolution/ high mass accuracy MS/MS experiments in an "all ion fragmentation" mode.

In fact, the Exactive mass spectrometer allows high efficiency "all ion fragmentation" experiments by means of Higher energy Collision induced Dissociation (HCD) [Olsen et al., 2007].

All these performance characteristics make this mass spectrometer well suited for discovery analyses, screening applications, quantitative estimation and elemental composition determination, solving the limitation of the ion trap technology and representing a good alternative to the ion trap based hybrid instruments.

5. Pharmacokinetic analysis

Pharmacokinetic and metabolic studies of *Ginkgo biloba* extracts concern the investigations of the main bioactive compounds, flavonoids and terpene-lactones.

Concerning flavonoids, few metabolic studies are available. In particular, Hackett (1986) described correlation between flavonoids and their metabolites using selected standard analyzed by means of Thin-layer chromatography (TLC). Of course, TLC technique didn't permit the investigation of complex extracts. Wang et al. (2003) developed a method based on liquid chromatography coupled to UV detector ($\lambda=380$ nm) for determining kaempferol and quercetin in human urine samples after orally administrated *Ginkgo biloba* extract.

Other authors used LC-UV and mass spectrometry for characterizing the metabolites of flavonoids in rat [Pietta et al, 1995] and human [Pietta et al., 1997] urine samples due to administration of standardized *Ginkgo biloba* extract. The main metabolite resulted to be conjugates of 4-hydroxybenzoic, vanillic and 4-hydroxyhippuric acids.

Recently, Ding et al. (2006) prepared an analytical method, based on ion trap mass spectrometry in negative ion mode, for assaying flavonoids in urine from volunteers after up-take with *Ginkgo biloba* extract. In addition, the authors monitored terpene-lactones simultaneously elution at a flowrate of 4 $\mu\text{L}/\text{min}$ (LOD around 2 and 10 ng/mL for flavonoids and terpenes, respectively). This method is interesting, but requires a long gradient (> 2 hours) and standard deviation resulted higher than 10%.

Concerning pharmacokinetics studies of terpene-lactones, many works are available. For example Biber [Biber & Koch; 1999] and co-workers [Furtillan et al.; 1995] used gas chromatography mass spectrometry for determining, after oral administration, the main ginkgolides in plasma from both humans [Furtillan et al., 1995] and rats [Biber & Koch, 1999]. In particular, in rats the half life of ginkgolides resulted to be around 2 h; at the contrary, in human differences were noted for ginkgolide A, ginkgolide B and bilobalide (4.5, 10.5, and 3.2 hours, respectively).

In 2001 Mauri et al. (2001) proposed a fast (within 8 min) method based on atmospheric pressure chemical ionization (APCI) interface coupled to an ion trap mass spectrometer to

monitor (LOD about 2 ng/mL) terpenes in plasma of volunteers after administration of two different *Ginkgo biloba* formulations (free and phospholipids complex formulations). When supplied in the phospholipid complex form, both C_{max} and AUC (Area Under the Curve) of terpene lactones increased, suggesting that this formulation may increase their bioavailability.

The same approach was extended to investigate pharmacokinetics of terpene-lactones in rats and guinea pigs after acute and chronic oral administrations [Mauri et al., 2003]. Other authors used APCI interface combined with a triple quadrupole analyzer for investigating the bioavailability of ginkgolides after intravenous administration to rats [Xie et al., 2008]. Specifically, multiple reaction monitoring (MRM) was used and the limit of quantification resulted around 2 ng/mL.

In alternative to APCI, Hua et al. (2006) proposed electrospray interface coupled to Q-array-Octapole-Quadrupole mass analyzer (QoQ) for investigating the intragastric administration of pure ginkgolide B (0.1 ng/kg) in Beagle dogs. The authors reported a LOD around 0.1 ng/mL while T_{max} and $t_{1/2}$ resulted to be 0.5 h and 2.8 h, respectively.

Other authors have studied the bioavailability of pure ginkgolide B after oral administration and T_{max} resulted around 2 and 4 h for phytosomic and free forms, respectively [Mauri et al., 2003].

Concerning ginkgolide C, different authors observed a very low recovery from plasma of this terpene-lactone. This is accompanied by the increase of methylated metabolite observed in plasma of both animals and humans [Mauri et al, 2006].

All in-vivo studies of terpene-lactones from *Ginkgo biloba* concern plasma or urine samples. However, very recently it has been published a study about the identification of bilobalide in rat brain after single oral dose [Rossi et al., 2009]. In particular, it has been observed that bilobalide presents different profiles in brain and plasma samples. In fact, in plasma the bilobalide levels increase with the administered dose; while the brain levels increase for dose up to 10 mg/kg; and decrease for higher doses. These results support the studies that described the positive cognitive efforts on brain due to *Ginkgo biloba* extracts (Lee et al., 2002; Kennedy et al., 2007). Moreover the absorption of bilobalide could be explained by a specific mechanism of transport and by an inhibition effect due to an overloading of transporter after its administration at high doses.

6. Conclusion

Ginkgo biloba contains mainly two types of constituents, the flavonoids and terpene lactones, which together have been proven to be responsible of the polyvalent activities of *Ginkgo biloba* herbal and *Ginkgo biloba*-containing preparations. In fact, for many centuries *Ginkgo biloba* was used for the treatment of several pathologies, but in recent years its interest increased in relation to the neuroprotective activities ascribed to terpene lactones. To this end, the development of many analytical technologies improved fingerprinting authentication and quantitative determination of target analytes, as well as the pharmacokinetic and pharmacodynamic studies on the active components of *Ginkgo biloba* and its finished products. This is because of selectivity and specificity achieved by both the chromatographic and mass spectrometry detection systems. In particular, LC-MS approach appeared to be the method of choice for the measurement of target analytes in biological samples. In fact, the separation efficiency and fastness of the new HPLC systems combined to the high resolution

and accurate measurements of the recent mass spectrometry detectors permit an unequivocal characterization of bioactive compounds from *Ginkgo biloba* extracts. These analytical tools will be very important for elucidating the transport and pharmacological *in vivo* mechanisms of terpene lactones and flavonoids.

7. References

- Ahlemeyer, B.; Mowes, A. & Krieglstein, J. (1999). Inhibition of serum deprivation- and staurosporine-induced neuronal apoptosis by Ginkgo biloba extract and some of its constituents. *Eur. J. Pharmacol.*, Vol. 367, N. 2-3, (Feb 1999), 423-430.
- Ahlemeyer, B. & Krieglstein, J. (2003). Pharmacological studies supporting the therapeutic use of Ginkgo biloba extract for Alzheimer's disease. *Pharmacopsychiatry*, Vol. 36, N. Suppl. 1, (Jun 2003), S8-S14.
- Ahlemeyer, B., Junker, V., Huhne, R. & Krieglstein, J. (2001). Neuroprotective effects of NV-31, a bilobalide-derived compound: evidence for an antioxidative mechanism. *Brain Res.*, Vol. 890, N. 2, (Feb 2001), 338-342.
- Ahmad, M., Saleem, S., Ahmad, A.S., Yousuf, S., Ansari, M.A., Kan, M.B., Ishrat, T., Chaturvedi, R.K., Agrawal, A.K. & Islam F. (2005). Ginkgo biloba affords dose-dependent protection against 6-hydroxydopamine-induced parkinsonism in rats: neurobehavioural, neurochemical and immunohistochemical evidences. *J. Neurochem.*, Vol. 93, N. 1, (Apr 2005), 94-104.
- Augustin, S., Rimbach, G., Augustin, K., Schliebs, R., Wolffram, S. & Cermak, R. (2009). Effect of a short- and long-term treatment with Ginkgo biloba extract on amyloid precursor protein levels in a transgenic mouse model relevant to Alzheimer's disease. *Arch. Biochem. Biophys.*, Vol. 481, N. 2, (Jan 2009), 177-182.
- Beal, M.F. (2003). Bioenergetic approaches for neuroprotection in Parkinson's disease. *Ann. Neurol.*, Vol. 53, N. Suppl 3, (2003), S39-S47.
- Biber, A. & Koch, E. (1999). Bioavailability of ginkgolides and bilobalide from extracts of ginkgo biloba using GC/MS. *Planta Med.*, Vol. 65, N. 2, (Mar 1999), 192-193.
- Blecharz-Klin, K., Piechal, A., Joniec, I., Pyrzanowska, J. & Widy-Tyszkiewicz, E. (2009). Pharmacological and biochemical effects of Ginkgo biloba extract on learning memory consolidation and motor activity in old rats. *Acta Neurobiol. Exp. (Wars)*, Vol. 69, N. 2, (2009), 217-231.
- Blumenthal, M., Goldberg, A. & Brinckman, J. (2000). Herbal medicine expanded commission E monographs. Integrative medicine Communications, Boston, Massachusetts. American Botany Council, Austin, TX, USA, 160-169.
- Blumenthal, M. (2000). Herb Sales Down 15 percent in Mainstream Market. *HerbalGram.*, Vol. 51, (Winter 2000), 69.
- Braquet, P (1987). The ginkgolides: potent platelet-activating factor antagonists isolated from Ginkgo biloba L.: chemistry, pharmacology and clinical applications. *Drug of the Future*, Vol, 12, N. 7, (1987), 643-699.
- Bridi, R., Crossetti, F.P., Steffen, V.M. & Henriques, A.T. (2001). The antioxidant activity of standardized extract of Ginkgo biloba (EGb 761) in rats. *Phytother. Res.*, Vol. 15, N. 5, (Aug 2001), 449-451.

- Bruno, C., Cuppini, R., Sartini, S., Cecchini, T., Ambrogini, P. & Bombardelli, E. (1993). Regeneration of motor nerves in bilobalide-treated rats. *Planta Med.*, Vol. 59, N. 4, (Aug 1993), 302-307.
- Caponovo, F.F., Wolfender, J.L., Maillard, M.P.M, Potterat, O. & Hostettmann, K. (1995). Evaporative light scattering and thermospray mass spectrometry: Two alternative methods for detection and quantitative liquid chromatographic determination of ginkgolides and bilobalide in *Ginkgo biloba* leaf extracts and phytopharmaceuticals. *Phytochemical Analysis*, Vol. 6, N. 3, (May-Jun 1995), 141-148.
- Chandrasekaran, K., Mehrabian, Z., Spinnewyn, B., Drieu, K. & Fiskum, G. (2001). Neuroprotective effects of bilobalide, a component of the *Ginkgo biloba* extract (EGb 761), in gerbil global brain ischemia. *Brain Res.*, Vol. 922, N. 2, (Dec 2001), 282-292.
- Chauret, N., Carrier, J., Mancini, M., Neufeld, R., Weber, M. & Archambault, J. (1991). Gas chromatographic-mass spectrometric analysis of ginkgolides produced by *Ginkgo biloba* cell culture. *J. Chromatogr.*, Vol. 588, N. 1-2, (Dec 1991), 281-287.
- Chavez, M.L & Chavez, P.I. (1998). *Ginkgo* (part 1): history, use, and pharmacologic properties. *Hosp. Pharm.*, Vol 33, (1998), 658-672.
- Chen, E., Ding, C. & Lindsay, R.C. (2005). Qualitative and quantitative analyses of ginkgo terpene trilactones by liquid chromatography/sonic spray ionization ion trap mass spectrometry. *Anal Chem.*, Vol. 77, N. 9, (May 2005), 2966-2970.
- Chen, L.W., Wang, Y.Q., Wie, L.C., Shi, M. & Chan, Y.S. (2007). Chinese herbs and herbal extracts for neuroprotection of dopaminergic neurons and potential therapeutic treatment of Parkinson's disease. *CNS Neurol. Disord. Drug Targets*, Vol. 6, N. 4, (Aug 2007), 273-281.
- Chen, P., Su, X. L., Nie, L. H., Yao, S. Z. & Zeng J. G. (1998). Analysis of Ginkgolides and Bilobalides in *Ginkgo biloba* L. Extract for Its Production Process Control by High-Performance Liquid Chromatography. *J. Chrom. Sci.*, Vol. 36, N.4, (1998), 197-200.
- Dajas, F., Rivera, F., Blasina, F., Arredondo, F., Echeverry, C., Lafon, L., Morquio, A. & Heizen, H. (2003). Cell culture protection and in vivo neuroprotective capacity of flavonoids. *Neurotox. Res.*, Vol. 5, N. 6, (2003) 425-432.
- Defeudis, F.V. (2002). Bilobalide and neuroprotection. *Pharmacol. Res.*, Vol. 46, N. 6, (Dec 2002), 565-568.
- Deng, F. & Zito, S.W. (2003). Development and validation of a gas chromatographic-mass spectrometric method for simultaneous identification and quantification of marker compounds including bilobalide, ginkgolides and flavonoids in *Ginkgo biloba* L. extract and pharmaceutical preparations. *J. Chromatogr A*, Vol. 986, N. 1, (Jan 2003), 121-127.
- Diamond, B.J., Schiflett, S.C., Fiewel, N., Matheis, R.J., Noskin, O., Richards, J.A. & Schoenberger, N.E. (2000). *Ginkgo biloba* extract: mechanisms and clinical indications. *Arch. Phys. Med. Rehabil.*, Vol. 81, N. 5, (May 2000), 668-678.
- Ding, S., Dudley, E., Chen, L., Plummer, S., Tang, J., Newton, R.P. & Brenton, A.G. (2006). Determination of active components of *Ginkgo biloba* in human urine by capillary high-performance liquid chromatography/mass spectrometry with on-line

- column-switching purification. *Rapid Commun Mass Spectrom.*, Vol. 20, N. 24, (Dec 2006), 3619-3624.
- Ding, S., Dudley, E., Plummer, S., Tang, J., Newton, R.P. & Brenton, A.G. (2008a). Fingerprint profile of Ginkgo biloba nutritional supplements by LC/ESI-MS/MS. *Phytochemistry*, Vol. 69, Vol. 7, (Mar 2008), 1555-1564.
- Ding, S., Dudley, E., Song, Q., Plummer, S., Tang, J., Newton, R.P. & Brenton A.G. (2008b). Mass spectrometry analysis of terpene lactones in Ginkgo biloba. *Rapid Commun. Mass Spectrom.*, Vol. 22, N. 6, (Mar 2008), 766-772.
- Domorakova, I., Mechirova, E., Dankova, M., Danielisova, V. & Burda, J. (2009). Effect of antioxidant treatment in global ischemia and ischemic postconditioning in the rat hippocampus. *Cell. Mol. Neurobiol.*, Vol. 29, N. 6-7, (Sep 2009), 837-844.
- Dubber, M.J. & Kanfer, I. (2006). Determination of terpene trilactones in Ginkgo biloba solid oral dosage forms using HPLC with evaporative light scattering detection. *J. Pharm. Biomed. Anal.*, Vol. 41, N. 1, (Apr 2006), 135-140.
- Ellnain-Wojtaszek, M., Kruczynski, Z. & Kasprzak, J. (2003) Investigation of the free radical scavenging activity of Ginkgo biloba L. leaves. *Fitoterapia*, Vol. 74, N.1-2, (Feb 2003), 1-6.
- Ernest, E. (2000). The role of complementary and alternative medicine. *Br. Med. J.*, Vol. 321, N. 7269, (Nov 2000), 1133-1135.
- Furtillan, G.J., Brisson, A.M., Girault, J., Ingrand, I., Decourt, J.P., Drieu, K., Jouenne, P. & Biber, A. (1995). Pharmacokinetic properties of Bilobalide and Ginkgolides A and B in healthy subjects after intravenous and oral administration of Ginkgo biloba extract (EGb 761). *Therapie*, Vol. 50, N. 2, (Mar-Apr 1995), 137-144.
- Furukawa, S. (1932). Constituents of Ginkgo biloba leaves. *Scientific Papers of the Institute of Physical and Chemical Research*, Vol. 19, (1932), 27.
- Goh, L.M., Barlow, P.J. & Yong, C.S. (2003). Examination of antioxidant activity of Ginkgo biloba leaf infusions. *Food chem.*, Vol. 82, N. 2, (2003), 275-282.
- Gohil, K. & Packer L. (2002). Global gene expression analysis identifies cell and tissue specific actions of Ginkgo biloba extract, EGb 761. *Cell. Mol. Biol.*, Vol. 48, N. 6, (Sep 2002), 625-631.
- Gong, Q.H., Wu, Q., Huang, X.N., Sun, A.S., Nie, J. & Shi, J.S. (2006). Protective effect of Ginkgo biloba leaf extract on learning and memory deficit induced by aluminium in model rats. *Chin. J. Integ. Med.*, Vol. 12, N. 1, (Mar 2006), 37-41.
- Ha, S.W., Yi, C.J., Cho, C.K., Cho, M.J., Shin, K.H. & Park, C.I. (1996). Enhancement of radiation effect by Ginkgo biloba extract in C3H mouse fibrosarcoma. *Radiother Oncol.* Vol. 41, N. 2, (Nov 1996), 163-167.
- Hackett, A.M. (1986). The metabolism of flavonoid compounds in mammals. In *Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological and Structure-Activity Relationship*, Alan R. Liss, New York, 177-194.
- Hasler, A., Sticher, O. & Meier, B. (1992). Identification and determination of the flavonoids from Ginkgo biloba by high-performance liquid chromatography. *J Chromatogr A*, Vol. 605, N. 1, (Jul 1992), 41-48.
- Hu, L., Chen, Z., Cheng, X. & Xie, Y. (1999). Chemistry of ginkgolides: structure-activity relationship as PAF antagonists. *Pure Appl. Chem.*, Vol. 71, N. 6, (1999), 1153-1156.

- Hua, L., Guangji, W., Hao, L., Minwen, H., Haitang, X., Chenrong, H., Jianguo, S. & Tian, L. (2006). Sensitive and selective liquid chromatography-electrospray ionization mass spectrometry analysis of ginkgolide B in dog plasma. *J. Pharm. Biomed. Anal.*, Vol. 40, N. 1, (Jan 2006), 88-94.
- Huch, H. & Staba E. (1993). Ontogenic Aspects of Ginkgolide Production in *Ginkgo biloba*. *Planta med.*, Vol. 59, N. 3, (Jun 1993), 232-239.
- Itokawa, H., Totsuka, N., Nakahara, K., Maezuru, M., Takeya, K., Kondo, M., Inamatsu, M. & Morita, H. (1989). A quantitative structure-activity relationship for antitumor activity of long-chain phenols from *Ginkgo biloba* L. *Chem Pharm Bull (Tokyo)*, Vol. 37, N. 6, (Jun 1989), 1619-1621.
- Janssens, D., Micheils, C., Delaive, E., Eliaers, F., Drieu, K. & Ramacle, J. (1995). Protection of hypoxia-induced ATP decrease in endothelial cell by *Ginkgo biloba* extract and bilobalide. *Biochem. Pharmacol.*, Vol. 50, N. 7, (Sep 1995), 991-999.
- Jensen, A.G., Ndjoko, K., Wolfender, J.L., Hostettmann, K., Camponovo, F. & Soldati, F. (2002). Liquid chromatography-atmospheric pressure chemical ionisation/mass spectrometry: a rapid and selective method for the quantitative determination of ginkgolides and bilobalide in ginkgo leaf extracts and phytopharmaceuticals. *Phytochem Anal.*, Vol. 13, N.1, (Jan-Feb 2002), 31-38.
- Jin, C.H., Shin, E.J., Park, J.B., Jang, C.G., Li, Z., Kim, M.S., Koo, K.H., Yoon, H.J., Park, S.J., Choi, W.C., Yamada, K. Nabeshima, T. & Kim, H.C. (2009). Fustin flavonoid attenuates beta-amyloid (1-42)-induced learning impairment. *J. Neurosci. Res.*, Vol. 87, N. 16, (Dec 2009), 3658-3670.
- Kandaswami, C., Lee, L.T., Lee, P.P., Hwang, J.J., Ke, F.C., Huang, Y.T. & Lee, M.T. (2005). The antitumor activities of flavonoids. *In Vivo*, Vol. 19, N. 5, (Sep-Oct 2005), 895-909.
- Kanowski, S., Herrmann, W.M., Stephan, K., Wierich, W. & Hörr, R. (1996). Proof of efficacy of the *Ginkgo biloba* special extract EGb 761 in outpatients suffering from mild to moderate primary degenerative dementia of the Alzheimer type or multi-infarct dementia. *Pharmacopsychiatry*, Vol. 29, N. 2, (Mar 1996), 47-56.
- Kennedy, D.O., Haskell, C.F., Mauri, P.L. & Sholey, A.B. (2007). Acute cognitive effects of standardised *Ginkgo biloba* extract complexed with phosphatidylserine. *Hum. Psychopharmacol.*, Vol. 22, N. 4, (Jun 2007), 199-210.
- Kiewert, C., Kumar, V., Hildmann, O., Hartmann, J., Hillert, M. & Klein, J. (2008). Role of glycine receptors and glycine release for the neuroprotective activity of bilobalide. *Brain Res.*, Vol. 1201, (Mar 2008), 143-150.
- Kiewert, C., Kumar, V., Hildmann, O., Rueda, M., Hartmann, J., Naik, R.S. & Klein, J. (2007). Role of GABAergic antagonism in the neuroprotective effects of bilobalide. *Brain Res.*, Vol. 1128, N. 1, (Jan 2007), 70-78.
- Kim, M.S., Lee, J.I., Lee, W.Y. & Kim S.E. (2004). Neuroprotective effect of *Ginkgo biloba* L. extract in a rat model of Parkinson's disease. *Phytother. Res.*, Vol. 18, N. 8, (Aug 2004) 663-666.
- Klein, J., Chatterjee, S.S. & Löffelholz, K. (1997). Phospholipid breakdown and choline release under hypoxic conditions: inhibition by bilobalide, a constituent of *ginkgo biloba*. *Brain Res.*, Vol. 755, N. 2, (May 1997), 347-350.

- Koch, E. (2005). Inhibition of platelet activating factor (PAF)-induced aggregation of human thrombocytes by ginkgolides: considerations on possible bleeding complications after oral intake of Ginkgo biloba extracts. *Phytomed.*, Vol.12, N. 1-2, (Jan 2005), 10-16.
- Kuo, S.M. (1997). Dietary flavonoid and cancer prevention: evidence and potential mechanism. *Crit. Rev. Oncog.*, Vol. 8, N. 1 (Sep 1997), 47-69.
- Lang, Q. & Wai, M. (1999). An extraction method for determination of ginkgolides and bilobalide in Ginkgo leaf extracts. *Anal. Chem.*, Vol. 71, N. 14, (Jul. 1999), 2929-2933.
- Le Bars P.L., Kats, M.M, Berman, N., Itil, T.M., Freedman, A.F. & Schatzberg J. (1997). A placebo-controlled, double-blind, randomized trial of an extract of Ginkgo biloba for dementia. North American EGb Study Group. *J. Am Assoc.*, Vol. 278, N. 16, (Oct 1997), 1327-1332.
- Lee, E.J., Chen, H.Y., Wu, T.Y. & Maynard, K.L. (2002). Acute administration of Ginkgo biloba extract (EGb 761) affords neuroprotection against permanent and transient focal cerebral ischemia in Sprague-Dawley rats. *J. Neurosci. Res.*, Vol. 68, N. 5, (Jun 2002), 636-645.
- Li, W. & Fitzloff, J.F. (2002a). HPLC determination of flavonoids and terpene lactones in commercial Ginkgo biloba products. *J. Liq. Chrom. Rel. Technol.*, Vol. 25, N. 16, (2002), 2501-2514.
- Li, W. & Fitzloff, J.F. (2002b). Simultaneous determination of terpene lactones and flavonoid aglycones in Ginkgo biloba by high-performance liquid chromatography with evaporative light scattering detection. *J. Pharm Biomed. Anal.*, Vol. 30, N. 1, (Aug 2002), 67-75.
- Liang, X., Zhang, L., Zhang, X., Dai, W., Li, H., Hu, L., Liu, H., Su, J. & Zhang, W. (2010). Qualitative and quantitative analysis of traditional Chinese medicine Niu Huang Jie Du Pill using ultra performance liquid chromatography coupled with tunable UV detector and rapid resolution liquid chromatography coupled with time-of-flight tandem mass spectrometry. *J. Pharm. Biomed. Anal.*, Vol. 51, N. 3, (Feb 2010), 565-571.
- Luo, Y. (2001). Ginkgo biloba neuroprotection: Therapeutic implication in Alzheimer's disease. *J. Alzheimers Dis.*, Vol. 3, N. 4, (Aug 2001), 401-407.
- Luo, Y. (2006) Alzheimer's disease, the nematode *Caenorhabditis elegans*, and Ginkgo biloba leaf extract. *Life Sci.*, Vol. 78, N. 18, (Mar 2006), 2066-2072.
- Luo, Y., Smith, J.V., Paramasivam, V., Burdick, A., Curry, K.J., Buford, J.P., Khan, I., Netzer, W.J., Xu, H. & Butko, P. (2002). Inhibition of amyloid-beta aggregation and caspase-3 activation by the Ginkgo biloba extract EGb761. *Proc Natl. Acad. Sci. USA.*, Vol. 99, N. 19, (Sep 2002), 12197-12202.
- Lynch, J.W. & Chen, X. (2008). Subunit-specific potentiation of recombinant glycine receptors by NV-31, a bilobalide-derived compound. *Neurosci. Lett.*, Vol. 435, N. 2, (Apr 2008), 147-151.
- Mahadevan, S. & Park., Y.(2008). Multifaceted Therapeutic Benefits of Ginkgo biloba L.: Chemistry, Efficacy, Safety, and Uses. *J. Food Sci.*, Vol. 73, N. 1, (Jan 2008), R14-19.
- Makarov, A., Denisov, E. & Lange O. (2009). Performance evaluation of a high-field Orbitrap mass analyzer. *J. Am. Soc. Mass Spectrom.*, Vol. 20, N. 8, (Aug 2009), 1391-1396.

- Mauri, P., Migliazza, B. & Pietta, P. (1999). Liquid chromatography/electrospray mass spectrometry of bioactive terpenoids in *Ginkgo biloba* L. *J. Mass Spectrom.*, Vol. 34, N. 12, (Dec 1999), 1361-1367.
- Mauri, P., Simonetti, P., Gardana, C., Minoggio, M., Morazzoni, P., Bombardelli, E. & Pietta, P. (2001). Liquid chromatography/atmospheric pressure chemical ionization mass spectrometry of terpene lactones in plasma of volunteers dosed with *Ginkgo biloba* L. extracts. *Rapid Commun. Mass Spectrom.*, Vol. 15, N. 12, (May 2001), 929-934.
- Mauri, P.L., Minoggio, M., Iemoli, L., Rossoni, G., Morazzoni, P., Bombardelli, E. & Pietta P.G. (2003). Liquid chromatography/atmospheric pressure chemical ionization ion trap mass spectrometry of terpene lactones in plasma of animals. *J. Pharm. Biomed. Anal.*, Vol. 32, N. 4-5, (Aug 2003), 633-639.
- Mauri, P.L., De Palma, A., Pozzi, F., Basilio, F., Riva, A., Morazzoni, P., Bombardelli, E. & Rossoni, G. (2006). LC-MS characterization of terpene lactones in plasma of experimental animals treated with *Ginkgo biloba* extracts: Correlation with pharmacological activity. *J. Pharm. Biomed. Anal.*, Vol. 40, N. 3, (Feb 2006), 763-768.
- Mdzinarishvili, A., Kiewert, C., Kumar, V., Hillert, M. & Klein, J. (2007). Bilobalide prevents ischemia-induced edema formation in vitro and in vivo. *Neuroscience*, Vol. 144, N. 1, (Jan 2007), 217-222.
- Naik, S.R., Pilgaonkar, V.W. & Panda, V.S. (2006). Evaluation of antioxidant activity of *Ginkgo biloba* phytosomes in rat brain. *Phytoter. Res.*, Vol. 20, N. 11, (Nov 2006), 1013-1016.
- Nakanishi, K., Habaguchi, K., Nakadaira, Y., Woods, M.C., Maruyama, M., Major, R.T., Alauddin, M., Patel, A.R., Weinges, K. & Bahr, W. (1971) Structure of bilobalide, a rare tert-butyl containing sesquiterpenoid related to the C20-ginkgolides. *J Am Chem Soc*, Vol. 93, (1971), 3544-3546.
- Olsen, J.V., Macek, B., Lange, O., Makarov, A., Horning, S. & Mann, M. (2007). Higher-energy C-trap dissociation for peptide modification analysis. *Nat. Methods.*, Vol. 4, N. 9, (Sep 2007), 709-712.
- Pietta, P., Facino R, Carini, M. & Mauri P. (1994). Thermospray liquid chromatography-mass spectrometry of flavonol glycosides from medicinal plants. *J. Chromatogr. A*, V. 661, N.1-2, (Feb 1994), 121-126.
- Pietta, P., Mauri, P. & Rava, A. (1992). Rapid liquid chromatography of terpenes in *Ginkgo biloba* L. extracts and products. *J. Pharm. Biomed. Anal.*, Vol. 10, N. 10-12, (Oct-Dec 1992), 1077-1079.
- Pietta, P.G., Gardana, C., Mauri, P.L., Maffei-Facino, R. & Carini, M. (1995). Identification of flavonoid metabolites after oral administration to rats of a *Ginkgo biloba* extract. *J. Chromatogr. B Biomed. Appl.*, Vol. 673, N. 1, (Nov 1995), 75-80.
- Pietta, P.G., Gardana, C. & Mauri, P.L. (1997). Identification of *Ginkgo biloba* flavonol metabolites after oral administration to humans. *J. Chromatogr B Biomed. Sci. Appl.*, Vol. 693, N. 1, (May 1997), 249-255.
- Pietta, P.G., Mauri, P.L. & Rava, A. (1990). Analysis of terpenes from *Ginkgo biloba* L. extracts by reversed phase high-performance liquid chromatography. *Chromatographia*, Vo.29, N. 5-6, (Mar 1990), 251-253.

- Rojas, P., Serrano-Garcia, N., Mares-Samano, J.J., Medina-Campos, O.S., Pedraza-Chaverri, J. & Ogren S.O. (2008). EGb761 protect against nigrostriatal dopaminergic neurotoxicity in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced Parkinsonism in mice: role of oxidative stress. *Eur. J. Neurosci.*, Vol. 28, N. 1, (Jul 2008), 41-50.
- Rossi, R., Basilico, F., Rossoni, G., Riva, A., Morazzoni, P. & Mauri, P.L. (2009). Liquid chromatography/atmospheric pressure chemical ionization ion trap mass spectrometry of bilobalide in plasma and brain of rats after oral administration of its phospholipidic complex. *J. Pharm. Biomed. Anal.*, Vol. 50, N.2, (Sep 2009), 224-227.
- Ryu, K.H., Han, H.Y., Lee, S.Y., Jeon, S.D., Im, G.J., Lee, B.Y., Kim, K., Lim, K.M. & Chung, J.H. (2009). Ginkgo biloba extract enhances antiplatelet and antithrombotic effects of cilostazol without prolongation of bleeding time. *Thromb Res. Jul*, Vol. 124, N. 3, (Apr 2009), 328-334.
- Saija, A., Scalsese, M., Lanza, M., Marzullo, D., Bonina, F. & Castelli, F. (1995). Flavonoid as antioxidant agents: importance of their interaction with biomembrane. *Free Radic. Biol. Med.*, Vol. 19, N. 4, (Oct 1995), 481-486.
- Sasaki, K., Hatta, S., Wada, K., Ohshika, H. & Haga, M. (2000). Bilobalide prevents reduction of gamma-aminobutyric acid levels and glutamic acid decarboxylase activity induced by 4-O-methylpyridoxine in mouse hippocampus. *Life Sci.*, Vol. 67, N. 6, (Jun 2000), 705-715.
- Schmid, W. (1997). Ginkgo thrives. *Nature*, Vol. 386, N. 6627, (Apr 1997), 755.
- Sener, G., Sehirili, O., Tozan, A., Velioglu-Ovunç, A., Gedik, N. & Omurtag, G.Z. (2007). Ginkgo biloba extract protects against mercury(II)-induced oxidative tissue damage in rats. *Food Chem. Toxicol.*, Vol. 45, N. 4, (Apr 2007) 543-550.
- Sharma, H.S. Drieu, K., alm, P. & Westman, J. (2000). Role of nitric oxide in blood -brain barrier permeability, brain edema and cell damage following hyperthermic brain injury. An experimental study using EGB-761 and Ginkgolide B pretreatment in the rat. *Acta neurochir. Suppl.*, Vol. 76, (2000), 81-86.
- Shi, C., Fang, L., Yew, D.T., Yao, Z., & Xu, J. (2010a). Ginkgo biloba extract EGb761 protects against mitochondrial dysfunction in platelets and hippocampi in ovariectomized rats. *Platelets*, Vol. 21, N. 1, (Feb 2010), 53-59.
- Shi, C., Xiao, S., Liu, J., Guo, K., Wu, F., Yew, D.T. & Xu, J. (2010b). Ginkgo biloba extract EGb761 protects against aging-associated mitochondrial dysfunction in platelets and hippocampi of SAMP8 mice. *Platelets*, Vol. 21, N. 5, (2010b), 379-379.
- Singh, B., Kaur, P., Gopichand, R.D. & Ahuja, P.S. (2008). Biology and chemistry of Ginkgo biloba. *Fitoterapia*, Vol. 79, N. 6, (Sep 2008), 401-418.
- Sloley, B.D., Urichuk, L.J., Morley, P., Durkin, J., Shan, j.J., Pang, P.K. & Coutts, R.T. (2000). Identification of kaempferol as a monoamine oxidase inhibitor and potential Neuroprotectant in extracts of Ginkgo biloba. *J. Pharm. Pharmacol.*, Vol. 52, N. 4, (Apr 2000), 451-459.
- Smith, J.V. & Luo Y. (2004). Studies on molecular mechanisms of Ginkgo biloba extract. *Appl Microbiol Biotechnol.*, Vol. 64, N. 4, (May 2004), 465-472.
- Smith, P.F., Maclennan, K. & Darlington, C. L. (1996). The neuroprotective properties of the Ginkgo biloba leaf: a review of the possible relationship to platelet-activating factor (PAF). *J. Ethnopharmacol.*, Vol. 50, N. 3, (Mar 1996), 131-139.

- Sticher, O. (1993). Quality of Ginkgo preparations. *Planta Med.*, Vol. 59, N. 1, (Feb 1993), 2-11.
- Sticher, O. (1999). Herbal Medicines for Neuropsychiatric Diseases, Ginkgo Preparations: Biochemical, Pharmaceutical, and Medical Perspectives, in: S. Kanba, E. Richelson (Eds.) *Seiwa Shoten Publishers*, Tokyo, 83.
- Stiker, O., Meier, B. & Hasler, A. (2000). The analysis of ginkgo flavonoids, in van Beek TA (ed), *Ginkgo biloba*. Harwood, Amsterdam, 179-202.
- Sun, Y., Li, W., Fitzloff, J.F. & Van Breemen, R.B. (2005). Liquid chromatography/electrospray tandem mass spectrometry of terpenoid lactones in *Ginkgo biloba*. *J. Mass Spectrom.*, Vol. 40, N. 3, (Mar 2005), 373-379.
- Tang, C., Wei, X. & Yin C. (2003). Analysis of ginkgolides and bilobalide in *Ginkgo biloba* L. extract injections by high-performance liquid chromatography with evaporative light scattering detection. *J. Pharm. Biomed. Anal.*, Vol. 33, N. 4, (Nov2003), 811-817.
- Urikova, A., Babusikova, E., Dobrota, A, Drgova, A., Kaplan, P., Tatarkova, Z. & Lehotsky, J. (2006). Impact of *Ginkgo Biloba* Extract EGb 761 on ischemia/reperfusion – induced oxidative stress products formation in rat forebrain. *Cell. Mol. Neurobiol.*, Vol. 26, N. 7-8, (Oct-Nov 2006), 1343-1353.
- Van Beek, T.A. & Montoro, P. (2009). Chemical analysis and quality control of *Ginkgo biloba* leaves, extracts, and phytopharmaceuticals. *J. Chromatogr. A.*, Vol.1216, N. 11, (Mar 2009), 2002-2032
- Van Beek, T.A. (2002). Chemical analysis of *Ginkgo biloba* leaves and extracts. *J Chromatogr. A.*, Vo. 967, N. 1, (Aug 2002), 21-55.
- Van Beek, T.A. (2005). Ginkgolides and bilobalide: their physical, chromatographic and spectroscopic properties. *Bioorg. Med. Chem.*, Vol. 13, N. 17, (Sep 2005), 5001-5012.
- Van Beek, T.A., Scheeren, H.A., Rantio, T., Melger, W.C. & Lelyveld, G.P. (1991). Determination of Ginkgolides and Bilobalide in *Ginkgo biloba* Leaves and Phytopharmaceuticals. *J. Chromatography*, Vol. 543, (1991), 375-387.
- Wang, F.M., Yao, T.W. & Zeng, S. (2003). Determination of quercetin and kaempferol in human urine after orally administrated tablet of ginkgo biloba extract by HPLC. *J. Pharm. Biomed. Anal.*, Vol. 13; N. 2, (Sep. 2003), 317-321.
- Wang, G.X., Cao, F.L & Chen, J. (2006). Progress in researches on the pharmaceutical mechanism and clinical application on *Ginkgo biloba* extract on various kinds of diseases. *Chin. J. Integr. Med.*, Vol. 12, N. 3, (Sep 2006), 234-239.
- Wang, H.F. & Ju, X.R. (2000). Rapid analysis of terpene lactones in extract of *Ginkgo biloba* L. by high performance liquid chromatography. *Se Pu*, Vol. 15, N. 5, (Sep 2000), 394-397.
- Wang, Y., Sheng, L.S. & Lou, F.C. (2001). Analysis and structure identification of trace constituent in the total ginkgolide by using LC/DAD/ESI/MS. *Acta Pharmacol. Sin.*, Vol. 36 N. 8, (Aug 2001) 606-608
- Weinges, K., Hepp, M. & Jaggy, H. (1987). Chemistry of ginkgolides. II. Isolation and structural elucidation of a new ginkgolide. *Liebigs Ann. Chem.*, Vol. 6, (1987), 521-526
- Xie, J., Ding, C., Ge, Q., Zhou, Z. & Zhi X. (2008). Simultaneous determination of ginkgolides A, B, C and bilobalide in plasma by LC-MS/MS and its application to the pharmacokinetic study of *Ginkgo biloba* extract in rats. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, Vol. 864, N. 1-2, (Mar 2008), 87-94.

- Yamamoto, Y., Adachi, Y., Fujii, Y. & Kamei C. (2007). Ginkgo biloba extract improves spatial memory in rats mainly but not exclusively via a histaminergic mechanism. *Brain Res.*, Vol. 1129, N. 9, (Jan 2007), 161-165.
- Yang, C., Xu, Y.R. & Yao, W.X. (2002). Extraction of Pharmaceutical Components from Ginkgo biloba Leaves Using Supercritical Carbon Dioxide. *J. Agric. Foods Chem.*, Vol 50, N. 4, (Feb 2002), 846-849.
- Yuan, C., Pan, J. & Hu, X. (2008). Determination of the derivative from ginkgolide B. *Nat. Prod. Res.*, Vol. 22, N. 15, (Oct 2008), 1333-1338.
- Zhou, W., Chai, H., Lin, P.H., Lumsden, A.B., Yao, Q. & Chen, C. (2004). Clinical use and molecular mechanism of action of extract of Ginkgo biloba leaves in cardiovascular diseases. *Cardiovascular. Drug Rev.*, Vol. 22, N. 4, (Winter 2004), 309-319.



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