Analysis of medicinal plant phenoloids by coupled tandem mass spectrometry

Ph.D. thesis

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Summary

Medicinal plant extracts and herbal preparations are complex mixtures of active- and ballast substances which may contain numerous, not infrequently up to several hundreds of different constituents with not exactly defined structures. Quality, safety and efficacy of these herbs is thus a great issue and their analysis is challenging. Tandem mass spectrometry coupled to high performance liquid chromatography (LC-MS/MS), as a sensitive, powerful and robust technique, capable of analysing very diverse complex liquid samples, may offer a solution.

The aim of our work was to revaluate traditionally well known, but less characterized herbs of various phenoloid content by the qualitative and quantitative analysis of their active substances, and to study the versatile capabilities of mass spectrometry in phytochemistry, and draw general conclusions regarding phytoanalytical mass spectral applications.

For the analyses of the methanolic and/or aqueous methanolic extracts of *Euphrasia rostkoviana* Hayne, *Satureja hortensis* L., *Filipendula ulmaria* L. MAXIM, *Sempervivum tectorum* L., and *Epilobium parviflorum* Schreb. LC-DAD-MS/MS methods were adopted and developed. Samples were analysed with a triple quadrupole analyzer with electrospray ionsource (ESI) in negative ion mode. For revealing the potential active components of *Euphrasia rostkoviana* a bioassay (*in vitro* antioxidant decolorization assay) guided extraction and fractionation was accomplished.

All measurements were accomplished with great selectivity and sensitivity at high throughput. LC coupled mass spectrometry served indeed as a universal analytical tool for the qualitative and quantitative analysis of phenolics from simple phenolic acids and salicylates towards more complex structures, like flavonoid glycosides and macrocyclic phenolics.

The single reaction monitoring (SRM) mode enabled the quantitation of the leading active substance of the *Euphrasia* sample, acteoside, at ppb level by great precision and accuracy. A total of seventeen different phenolic acids and flavonoid glycosides were identified or characterized in the extract and in its fractions [1, 2]. Quantification of the antioxidant rosmarinic acid in *Satureja hortensis* was not amenable nor in selected ion monitoring (SIM) nor in SRM mode due to non-quantitative dimer formation, thus UV based quantitation was performed. Contents of six salicylates were determined with salicin and salicylic acid standards in SIM mode in the *Filipendula ulmaria* sample, and several flavonoids were characterized [3]. A comprehensive LC-MS characterization of the glycosilation profile of the *Sempervivum* flavonoids was accomplished based upon rel. intensities of the fragment ions and radical fragments [4]. The simultaneous formation of the single $[M-H]^-$ and double $[M-2H]^{2-}$ charged molecular ion of oenothein B helped the interpretation of its fragmentation in the *Epilobium* sample [5]. However some limitations regarding constitutional isomeria and stereochemistry (sugar moieties) of mass spectrometry in phytochemistry were pointed out which, still, are of almost no significance if compared to the possibilities of the technique.

1. **Blazics B**, Ludányi K, Szarka Sz, Kéry Á. (2008) Investigation of *Euphrasia rostkoviana* Hayne Using GC-MS and LC-MS. Chromatographia 68: S119-S124.

2. **Blazics B**, Alberti Á, Béni Sz, Kursinszki L, Tölgyesi L, Kéry Á. Identification and LC-MS/MS determination of acteoside, the main antioxidant compound of *Euphrasia rostkoviana*, using the isolated target analyte as external standard. Accepted for publication in J Chrom Sci, in press.

3. **Blazics B**, Papp I, Kéry Á. Qualitative Analysis and Simultaneous Determination of Six *Filipendula* Salicylates with Two Standards by LC-MS. Accepted for publication in Chromatographia, in press.

4. Alberti Á, **Blazics B**, Kéry Á. (2008) Evaluation of *Sempervivum tectorum* L. flavonoids by LC and LC-MS. Chromatographia 68: S107-S111.

5. Hevesi Tóth B, **Blazics B**, Kéry Á. (2008) Polyphenol composition and antioxidant capacity of *Epilobium* species. J Pharm Biomed Anal 49: 26-31.

Összefoglalás

A gyógynövénykivonatok és növényi gyógyszerek mindig több, nem ritkán többszáz komponsű, kémiailag gyakran nem pontosan definiált szerkezetű ható- és ballasztanyagok keverékei. Mindez a növényi gyógyszer minőségi vizsgálatában és analitikai értékelésében komoly kihívást jelent. A nagyhatékonyságú folyadékkromatográfiával kapcsolt tandem tömegspektrometria (LC-MS/MS) érzékenységének, hatékonyságának és robosztusságának köszönhetően választ jelenthet a kihívásra, hiszen széleskörűen alkalmas komplex folyékony minták vizsgálatára.

Jelen munkánkat kettős célkitűzés vezette. Egyrészt hozzá kívántunk járulni néhány fenoloid tartalmú tradícionális gyógynövény újraértékeléséhez tartalmi/hatóanyagaik feltárásával és mennyiségi vizsgálatával, másrészt további bizonyítékokat, konklúziókat kívántunk szolgáltatni az LC-MS módszerek fitokémiai és fitoanalitikai lehetőségeinek megismeréséhez. Az *Euphrasia rostkoviana* Hayne, *Filipendula ulmaria* L. MAXIM, *Sempervivum tectorum* L., *Satureja hortensis* L. és az *Epilobium parviflorum* Schreb. metanolos és vizes-metanolos extraktumainak vizsgálatára LC-DAD-MS/MS módszereket adaptáltunk és fejlesztettünk ki. A vizsgálatokat elektroporlasztásos ionforrással (ESI) ellátott hármas kvadrupól tömegspektrométerrel végeztük negatív ionizációs üzemmódban. Az *Euphrasia rostkoviana* potenciális hatóanyagának feltárása céljából bioassay (szabadgyökfogó képesség *in vitro* dekolorizációs módszer) vezetett extrakciót és frakcionálást választottunk.

Vizsgálatainkat nagy érzékenység és jó szelektívitás mellett nagy áteresztőképességgel sikerült elvégeznünk. A kapcsolt tandem tömegspektrometria univerzális kvalitatív és kvantitatív analitikai eszköznek bizonyult a fenoloidok vizsgálatára az egyszerű fenolsavaktól a bonyolultabb flavonoid glikozidokon át a makrociklusos polifenolokig. A single reaction monitoring (SRM) üzemmód jó precízitás és pontosság mellett ppb szinten tette lehetővé az *Euphrasia* vezető antioxidáns hatóanyagának (akteozid) meghatározását. A szemvidítófű kivonatokban összesen tizenhét egyszerű fenolos komponenst és flavonoid glikozidot azonosítottunk és/vagy jellemeztünk. Antioxidáns hatásvizsgálataink alapján megállapítottuk, hogy a jelentős koncentrációban feldúsuló akteozid a szemvidítófű hatóanyagának tekinthető. [1, 2]. Kísérletesen igazoltuk a *Satureja* kivonatok példáján, hogy, egy vélhetően az ionorrásban végbemenő, nem-kvantitatív dimérképződés miatt az antioxidáns rozmaringsav kvantálása tömegspektrometriás detektálással sem selected ion monitoring (SIM), sem SRM módban nem végezhető el. Így meghatározását UV detektálással végeztük. Szalicin és szalicilsav standardek segítségével hat szalicilát mennyiségét határoztuk meg SIM üzemmódban, valamint számos flavonoidot azonosítottunk a *Filipendula* mintákban [3]. A fragmens ionok és a gyökös fragmens ionok relatív intenzitásarányai alapján sikerrel térképeztük fel számos *Sempervivum* flavonoid glikozilációs profilját, a cukrok kapcsolódási helyét és sorrendjét. [4]. Az egyszeres $[M-H]^-$ és kétszeres $[M-2H]^{2-}$ töltésű molekulaionok szimultán detektálása és fragmentációja alapján elsőként írtuk le az oenothein B makrociklusos fenoloid tömegspektrometriás fragmentációját [5]. A változatos szerkezetű fenoloidok LC-MS/MS adattárának bővítése mellett rámutattunk néhány, a módszer előnyeihez képest nem jelentős hátrányra is (pl: izoméria).

1. **Blazics B**, Ludányi K, Szarka Sz, Kéry Á. (2008) Investigation of *Euphrasia rostkoviana* Hayne Using GC-MS and LC-MS. Chromatographia 68: S119-S124.

2. **Blazics B**, Alberti Á, Béni Sz, Kursinszki L, Tölgyesi L, Kéry Á. Identification and LC-MS/MS determination of acteoside, the main antioxidant compound of *Euphrasia rostkoviana*, using the isolated target analyte as external standard. Közlésre elfogadva J Chrom Sci, nyomdában.

3. **Blazics B**, Papp I, Kéry Á. Qualitative Analysis and Simultaneous Determination of Six *Filipendula* Salicylates with Two Standards by LC-MS. Közlésre elfogadva Chromatographia, nyomdában.

4. Alberti Á, **Blazics B**, Kéry Á. (2008) Evaluation of *Sempervivum tectorum* L. flavonoids by LC and LC-MS. Chromatographia 68: S107-S111.

5. Hevesi Tóth B, **Blazics B**, Kéry Á. (2008) Polyphenol composition and antioxidant capacity of *Epilobium* species. J Pharm Biomed Anal 49: 26-31.

Introduction

Nature, and natural compounds have an unexceptional influence on pharma R & D as they provide an uncountable number of invaluable lead molecules. Phytochemical researches of nowadays focus on bio-assay guided revealing of the therapeutic profile and synergism of medicinal herbs and their constituents. Assesing the clinical and biological potential and determining the pharmacokinetics of herbal constituents is also a hot area.

Identification and determination of the active substances (either major or minor) is a crucial precondition for the development of modern evidence-based phytomedicines according to the regulations of the EMEA and FDA. Medicinal plant extracts and herbal preparations are complex mixtures of active- and ballast substances which may contain not infrequently up to several hundreds of different constituents with not exactly defined structures. Hence chromatography is undoubtly fundamental to overcome the challenges of phytoanalytics. Regular HPLC associated detectors (UV, refractive index), are not selective and sensitive enough according to modern requirements. Mass spectrometry (MS) offers great selectivity and sensitivity and by coupling to high performance liquid chromatography (LC-MS) it enables effective analysis of complex matrices. For the analysis of volatile, rather apolar molecules gas chromatography-mass spectrometry (GC-MS) is the hyphenated method of choice. Since typical and potential drug molecules are rather polar and water soluble LC-MS has a greater significance than GC-MS. By tandem mass spectrometry (MS/MS) a full structural analysis of a mixture can be accomplished by a few runs involving no time-consuming isolation processes. Triple quadrupole MS/MS systems ensure excellent selectivity and sensitivity for quantitative aims. Nuclear magnetic resonance spectroscopy (NMR) serves as a complement analytical tool for LC-MS systems in unambiguous structure elucidation. Today LC-NMR-MS is perhaps the most promising hyphenation technique, but it still needs a few years time to be put in routine, not to talk about its stratospherical price, while LC-MS rapidly becomes a routine technique for the fast and powerful analysis of almost any complex matrix.

Aims

Two distinct, but strongly related aims governed our work. First, we aimed to revaluate the phytotherapeutical potential of selected, traditionally well known, but chemically less

characterized medicinal plants by the qualitative and quantitative analyses of their active substances. Modern LC-MS methods were applied to investigate the selected traditional herbs of various phenoloid content posessing significant medicinal effects.

Our second aim was to provide modern analytical solutions to replace older, less selective methods of phytoanalytics by studying the versatility and potential of coupled mass spectrometry in phytochemical applications, and draw general conclusion based upon our particular analyses.

Particular model plants were selected with the aim to represent the wide structural and therapeutical variety of phenolics from simple phenolic acids to macrocyclic polyphenols. Either active substance or ballast, phenolics are nearly always an issue in quality control and quality assurance of herbal products.

With the above mentioned motivation our investigations aimed to identify or characterize the active phenolic substances and determine the acteoside content of the traditional herbal eye-remedy, *Euphrasia rostkoviana* HAYNE (Eyebright) by the help of oriented antioxidant bio-assays.

We aimed to expand the scarce quantitative information on rosmarinic acid, the main antioxidant phenolic compound of *Satureja hortensis* (Savory).

Filipendula ulmaria L. (Meadowsweet) is a poorly characterized herb of serious antypiretic and anti-inflammatory salicylate content, therefore we aimed to analyse its phenolic constituents, characterize and quantify the salicylate components.

The flavonoid content of *Sempervivum tectorum* (Houseleek) is known partly and only at aglycon level. Since glycosilation status affects bioavalibility we aimed the characterization of the *Sempervivum* flavonoids on the glycoside level.

For providing detailed mass spectral information for the quality assurance of *Epilobium parviflorum* Schreb. (Willowherb) we aimed to characterize and interpret the fragmentation of oenothein B, a special macrocyclic phenolic of the herb.

Materials and methods

Plant materials

Cultivated, collected and commercially available plant samples were involved in our investigations: *Euphrasia rostkoviana* Hayne (herba, collected in Mures County, Romania, July 2006.), *Filipendula ulmaria* L. Maxim (herba and flos, collected around

Bükkszentkereszt, Hungary, June 2007.), *Sempervivum tectorum* L. (flos, cultivated at the Research Station of the Corvinus University of Budapest, Soroksár, 2008.), *Satureja hortensis* L. (herba, commercial sample), *Epilobium parviflorum* Schreb. (herba, collected in the Budai-hegység, Hungary, 2006.). Plant samples were authenticated in the Department of Pharmacognosy, Semmelweis University, Budapest, where the voucher specimens are deposited.

Extraction and sample preparation

All plant samples, except the *Epilobium*, were extracted with a Soxhlet extractor using methanol according to the instructions of the Ph. Hg. VIII. *Epilobium* sample was extracted with 80% (v/v%) acetone in an ultrasound sonicator.

For pre-separation and purification reasons samples (except *Filipendula* and *Satureja*) were subjected to an SPE procedure prior to analysis. Tubes (Supelclean LC-18 500 mg/3mL) were conditioned with methanol and with 2.5% (v/v%) acetic acid. Tubes were eluted in three steps, with 25%, 70% and 100% (v/v%) methanol. In cases of *Sempervivum* and *Epilobium* samples the first elution step of 25% was cancelled.

The *Euphrasiae herba* total extract was fractionated by conventional open column chromatography using polyamide as stationary phase. The column was eluted successively with water, aqueous-methanol and methanol. Isolation of the main compound of the *Euphrasia* sample was achieved also with polyamide column chromatography.

Antioxidant assay

The scavenging activity of the methanolic fractions of *Euphrasiae herba* was determined spectrophotometrically using free radicals of ABTS $^{\bullet+}$ and DPPH $^{\bullet}$ in a decolorization *in vitro* assay. Fractions were characterized by their IC_{50} value.

Nuclear magnetic resonance (NMR) analysis

NMR experiments of the *Euphrasia* isolate were carried out on a 600 MHz Varian VNMRS spectrometer equipped with a dual 5 mm inverse-detection gradient (IDPFG) probehead. Standard pulse sequences, as ^{1}H, ^{13}C, COSY, TOCSY, NOESY, HSQC, HMBC were applied.

High performance liquid chromatography - mass spectrometry (HPLC-MS)

All analyses were performed with an Agilent 6410 triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source in negative ion mode coupled to an

Agilent 1100 HPLC system. Accurate mass and elemental analysis was achieved by an Agilent 6210 time-of-flight mass spectrometer operating with a dual-nebulizer ESI source in the negative ion mode.

Reversed phase HPLC columns of C18 stationary phase were used for chromatography. Several gradient HPLC methods were developed according to differneces of samples including eluents of formic acid or acetic acid in water (A%) and methanol or acetonitril (B%). The QQQ analyzer and the UV detector were used for data acquisition simultaneously in all analayses. ESI parameters and collision energies were optimised before all quantitative analyses.

Identification of the components was carried out by the comprehensive interpretation of UV, scan and CID mass spectral data and by the comparison with those of literature data and authentic standards. Quantitative methods were validated (linearity, inter- and intra-day precision, accuracy, LOD, LLOQ, recovery), or at least all accesible method performance was tested.

Results and discussion

All of our aims were effectively accomplished at high sensitivity and great selectivity, the LC-MS/MS was proved to be a widely applicable and extra-ordinarly powerful analytical tool.

A number of qualitative and quantitative results were provided for the revaluation of several valuble tradtitionally known medicinal herbs.

Phytochemistry

Euphrasia rostkoviana Hayne

Eyebright herb is rich in flavonoids and other phenolics, results of contents are the following; flavonoids: 0.38 ± 0.03 g/100g (hyperoside), polyphenols: 1.47 ± 0.09 g/100g (pirogallol), tannins: 0.56g ± 0.04 /100g (pirogallol) and hydroxycinnamic derivatives: 1.97 ± 0.09 g/100g (rosmarinic acid). We were among the first to provide data on the antioxidant effect of the phenolics in *Euphrasia rostkoviana* Hayne. The methanolic fractions of the herb exhibited strong anti-radical activity in a concentration dependent way in both assays. Fraction I, characterized as a pure phenyl-propane compound, exhibited the strongest antioxidant effect (DPPH IC_{50}: 11.88 ± 0.39 μg/ml, ABTS IC_{50}: 4.24 ± 0.18 μg/ml). In general, fractions rich in

phenyl-propanes and rutin displayed the highest free-radical scavenging activity. The traditionally known anti-inflammatory effect of the multi-component eyebright extract may be, therefore partly attributed to its antioxidant phenolic constitutes. Drawing firm conclusion from these rapid antioxidant assays is hardly easy or relevant, however a rough overview of the scavenging activity can be gained and used as guidelines for further studies.

In order to find correlation between the antioxidant effect and quality, *Euphrasia* fractions were analysed by LC-DAD-MS/MS, LC-TOF and NMR. A total of seven simple phenolic acids and ten glycosides of luteolin, kaempferid, quercetin, apigenin and isorhamnetin flavonoid aglycons were identified and/or characterized. The chief constituent of the total extract, which was contained purely by fraction I, was charcterized as acteodside based on CID results and TOF elemental analysis. Identity was confirmed by NMR. In view of the significant antioxidant and other biological effects of acteoside we determined its content in the *Euphrasia* extract. The high isolation purity (≥ 97.1%) enabled us to use the acteoside isolate (yield: 51 mg) as standard for an external calibration quantitation.

The acteoside content of the herb was 2.56 ± 0.19 g / 100 g dry plant material (n = 3), which is prominently high, and may explain, in part, the beneficial effect of *Euphrasia* concerning inflamed eye-disorders. Validation results were well within widely accepted limits and ranges. According to our literature search, acteoside, the potential active component of the herb has neither been identified, nor quantified in *Euphrasia rostkoviana* before.

***Satureja hortensis* L.**

The leading phenolic antioxidant compound of *Satureja* was identified as rosmarinic acid by LC-DAD-MS/MS, and succesfully quantified by UV detection in different v/v% ethanolic extracts of the herb. Results of contents are the following: 50% EtOH extract: 4.69 extract % ± 0.02, 70% EtOH extract: 6.69 extract % ± 0.05, 96% EtOH extract: 6.47 extract % ± 0.09, 100% EtOH: 5.59 extract % ± 0.07. Due to the non-quantitative dimer formation of rosmarinic acid neither SIM nor SRM based determination was possible. Method performance was tested in a limited extent.

***Filipendula ulmaria* L.**

Six salicylates and a total number of fourteen phenolics, glycosides and aglycons of quercetine and kaempferol were identified and/or structurally characterized in the methanolic extracts of the herb and flower sample. Acetic acid was found improper for separation due to adduct formation. A switch to formic acid solved the problem, but only partly, since formate

adducts were present as well, however at a much lower occurance. Among salicylates salicylic acid, in free and glycoconjugated form, monotropitin and salicin were identified. Besides, two other salicylates were tentatively characterized as salicylalcohol derivatives based upon their UV and CID spectral data.

In light of their pharmacological significance we determined the content of salicin and salicylic acid by an external calibration method applying the corresponding standards. The remaining four salicylates were not commercially available as standards, thus their content was determined with the use of salicin and salicylic acid standard calibration by assuming similar ESI ionization and sensitivity. Therefore, results of salicylates with no standards are to be considered merely as estimative contents, though there is no way to gain more accurate results. Contents of different salicylates were within a range of 0.0002 ± 0.0001 and 8.36 ± 0.20 mg/1 g dry plant material (mean ± S.D.) in the herba and flower samples. Precision, accuracy and other validation results, except the recovery for salicin (54.1 %, RSD % = 9.43, n=3) were all within accepted ranges. Both the herb and flower sample contained all six salicylates, but in different ratios.

Such comprehensive phytoanalytical investigation of *Filipendula ulmaria* was not performed previously and our work is the first to provide quantitative data on any *Filipendula* salicylates.

***Sempervivum tectorum* L.**

Seven di-, tri- and tetra glycosides of kaempferol and quercetine were identified and tentatively characterized in the *Sempervivum* extract. The characterized flavonoid glycosides of high polarity may play significant role in the traditionally experienced effect of *Sempervivum* leaves. Glycosylation structure of *Sempervivum* flavonols was studied and described for the first time.

***Epilobium parviflorum* Schreb.**

We succesfully interpreted the mass spectral CID fragmentation of the macrocyclic tannin constituent, oenothein B for the first time. The analyte was detected simultaneously in single ($[M-H]^-$) and double ($[M-2H]^{2-}$) charged states. The different CID spectra of the two molecular ions served as complemets in structure elucidation. It was demonstraed by several studies that oenothein B shows a beneficial effect in benign prostate hyperplasia.

Conclusion

A total of seventeen simple phenolic acids and flavonoid glycosides were identified and/or structurally characterized in *Euphrasia rostkoviana* Hayne by LC-MS/MS. Among these one phenolic acid, one phenylpropane glycoside, one flavonoid aglycone and six flavonoid glycosides were reported in Eyebright for the first time. We isolated, characterized and identified acteoside, a widespread phenylpropane, by UV, MS and NMR spectroscopy for the first time in the species.

We were the first to quantify the acteoside content of *Euphrasia rostkoviana* by a validated, reliable and fast SRM method at ppm level.

According to the results of our *in vitro* antioxidant bio-assays it was concluded that acteoside is to be considered an active substance of Eyebright herb.

By the study of the ethanolic *Satureja* extracts it was proved that the MS based determination of rosmarinic acid is amenable neither in SIM, nor in SRM mode, which is due to a supposed non quantitative in-source dimer formation of the analyte. UV detection was thus concluded as the method of choice for the quantification of rosmarinic acid according to the available LC-MS system operated at the mentined settings.

Five flavonoid glycosides, three phenylpropane derivatives and three salicylates among the investigated ten flavonoids, four phenylpropanes and six saliclyates were reported in *Filipendula ulmaria* for the first time. This work is the first to determine the content of six salicylates in the herba and flower samples using salicin and salicylic acid standards in SIM mode. All contents were calculated and compared based on calibrations of dalicin and salicylic acid and the methods were validated. Our work provided results for understanding the undeservingly less known phytochemistry of herbal salicylates in view of their therapeutical significancy.

We were the first to accomplish the tentative characterization of the glycosilation profile of seven *Sempervivum* flavonoid di-, tri- and tetra glycosides based upon the relative intensities of fragments $(Y_0\text{-}2H)^{\cdot-}$, $(Y_0\text{-}H)^{\cdot-}$ and $(Y_0)^-$ formed via different CID mechanisms. Information

on the type and distribution of sugar units, the glycan sequence and the glycosilation position is of importance considering bioavailability.

Interpretation of the mass spectral fragmentation of the macrocyclic polyphenol, oenothein B, the active substance of *Epilobium parviflorumban*, was reported first. The structural elments were matched with the referring main fragments based upon the complementary investigation of the $[M-H]^-$ and $[M-2H]^{2-}$ pseudomolecular ions.

The rapidly reachable information density of the mass spectral results should be highlighted first among the numerous advantage, which makes coupled tandem mass spectrometry such a widely applicable exceptionally powerful analytical tool. Even a simple scan mass spectrum supplied with a wealth of information (eg.: acteoside, molar weight, isotopes, N content, C atom number). CID fragmentation provided valuable structural information, SIM and SRM modes ensured highly selective and sensitive determination. Mass spectrometry vs. UV detection served as an uncomparably efficient tool for interpreting co-elutions.
According to our particular studies the very few phytochemical limitations of the technique include the problem of isomeria (eg.: orto, meta or para coumaric acid in *Euphrasia* sample, stereochemistry of sugar moieties), the problematic distinction of structuraly similar isobar flavonoid glycosides of high molar weight (aglycon: kaempferol vs. luteolin, $C_{15}H_{10}O_6$ MW = 286 g/mol) and adduct formation (acetate and formate adducts) which rended difficulties in the analysis of the *Filipendula* samples.

List of publications fundamentally related to the thesis

Publication in journal

B. Blazics, Á. Kéry
Antioxidant activity of compounds in *Euphrasia officinalis* L. - revaluation of a traditional medicinal plant
Planta Medica (2007) 73: P_266 DOI: 10.1055/s-2007-987047

B. Blazics, K. Ludanyi, Sz. Szarka, A. Kery
Investigation of *Euphrasia rostkoviana* Hayne using GC-MS and LC-MS
Chromatographia (2008) 68: S119-S124

A. Alberti, **B. Blazics**, A. Kery
Evaluation of *Sempervivum tectorum* L. Flavonoids by HPLC and LC-MS Methods
Chromatographia (2008) 68: S107-S111

B. Hevesi Tóth, **B. Blazics**, Á. Kéry
Polyphenol composition and antioxidant capacity of *Epilobium* species
Journal of Pharmaceutical and Biomedical Analysis (2009) 49: 26-31

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Az *Euphrasia rostkoviana* Hayne fenoloid tartalmú frakcióinak antioxidáns értékelése
Acta Pharmaceutica Hungarica (2009) 79: 11-16.

B. Blazics, Á. Alberti, Sz. Béni, L. Kursinszki, L. Tölgyesi, Á. Kéry
Identification and LC-MS/MS determination of acteoside, the main antioxidant compound of *Euphrasia rostkoviana*, using the isolated target analyte as external standard
Journal of Chromatographic Science (2010) - accepted, in press

B. Blazics, I. Papp, Á. Kéry
Qualitative Analysis and Simultaneous Determination of Six *Filipendula* Salicylates with Two Standards by LC-MS
Chromatographia (2010) – accepted, in press

Oral presentation

Blazics B., Kéry Á.
Az *Euphrasia officinalis* L. (szemvidítófű) fenoloidjainak LC-MS/MS vizsgálata - Magyar Kémikusok Egyesülete, Fiatal Analitikusok Előadóülése, Budapest 2007. 11. 20.

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Az *Euphrasia rostkoviana* Hayne fitokémiai vizsgálata – Mozsonyi Sándor Alapítvány 20. Jubileumi Tudományos Ülés, Budapest, 2008. 04. 18.

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Tandem tömegspektrometriás tapasztalataink a fenoloidok/flavonoidok vizsgálatában – X. Gyógynövény Szimpózium, Pécs 2008. 10. 16-18.

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Tandem tömegspektrometriás tapasztalatok a gyógynövényanalitikában - lehetőségeken innen, korlátokon túl – Agilent LC-MS szeminárium, Budapest, 2008 10. 22.

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