UDC 582.71:582.943:543.54.4 https://doi.org/10.26577/2218-7979-2016-9-1-46-51

*Alimzhanova M., Makhayeva D.

Al-Farabi Kazakh National University,
Center of physico-chemical methods of research and analysis, Almaty, Kazakhstan
*e-mail: mereke.84@mail.ru

Identification of flavonoids in plant samples by gas chromatography-mass spectrometry with pre-derivatization

Abstract: Plants of Artemisia L. genus are important source of flavonoids – a group of biologically active substances, which are widely used in the perfume, alcoholic beverage, pharmaceutical industry and medicine. Flavonoids have antiseptic, anti-tumor, anti-inflammatory, antibacterial, antituberculosis actions. Therefore, testing and introduction of many types of Artemisia to the culture, and their selection in Kazakhstan is up-and-coming. Qualitative composition of wormwood Artemisia scopariaWaldst. et Kit flavonoids collected in Almaty and Ile-Balkhash region in 2013 year was studied by gas chromatography-mass spectrometry method. Samples were prepared by extraction three times with 40% ethanol solution. During the identification of flavonoids by gas chromatography-mass spectrometry pre-derivatization of the compounds was carried out by N,O-Bis(trimethylsilyl) trifluoroacetamide, which is the most reactive derivatizing agent to increase the volatility and thermal stabilityofstudied substances. Kaempferol and quercetin were found as a result of analysis.

Key words: analysis; Artemisia; derivatization; flavonoids; gas chromatography; kaempferol; mass spectrometry; quercetin.

Introduction

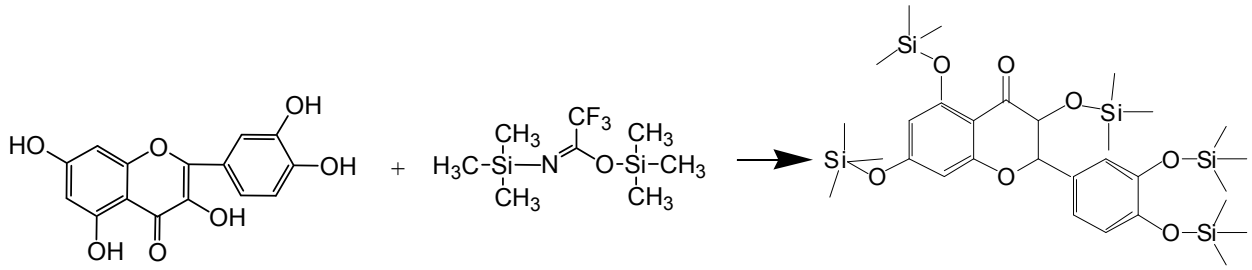
According to scientists [1], the linearity ranges of the measured concentrations of phenolic compounds in the analysis by GC-FID is considerably wider than when analyzing by GC-MS, but the limits of detection using mass spectrometric detector is 2-3 times lower. Scientists [2] used the technique of GC-FID described in work [3], and it was possible to identify a number of flavonol glycosides in bearberry extracts. Researchers [4] used capillary gas chromatography with flame ionization detector to assess the content of flavones, flavonones, flavonoles and isoflavones in legumes. According to the method [5] the content of isoflavones in soybean was determined, the authors were able to identify three aglyconesin plant sample:quercetin, genistein anddaidzein.

Gas chromatography-mass spectrometry is used to analyze the different objects. American scientists [6] had developed a technique of GC-MS with electron impact ionization for the analysis of biological fluids (human plasma, urine) on the content of flavonoids and phenolic acids after consumption of cranberry juice. In research [7] a method of determining the cinnamic acid, catechins and flavonols in red wines proposed.

A method for analyzing of olive oil on the content of phenolic compounds described in [8]. Detection of phenolic compounds was performedusing time-of-flight mass spectrometer with chemical ionization at atmospheric pressure. Detection limit for luteolin was 1.1 μg/g, for apigenin - 0.6 μg/g, for ferulic and coumaric acids - 0.2 μg/g. The authors of [9] carried out the analyses of the content of more than 20 different phenolic compounds and accompanying substances in aloe extract by GC-MS. Application of GC-MS in the analysis of mosses, herbs, fruits and vegetables is described in [10-12]. Most known methods of derivatization are alkylation and silylation. The mechanism of derivatization includes replacing the acidic hydrogen atom in the molecule of phenolic compound (for example, -OH and -COOH groups) by the alkyl or silyl group [13]. Various silylating agents: trimethylchlorosilane (TMCS), bis-trimethylsilylacetamide (BSA) dimethylsilylacetate (DMTSA) et al are used for derivatization. [7]. Among all the listed reagents N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) has the highest silylating ability which also modifies the surface of the sorbent column, which greatly improves the resolution of peaks. Pyridine can be added to speed up derivarization, because BSTFA slowly reactes with tertiary or quaternary hydroxyl groups [13]. In [7.10] pyridine is initially added to the solution of the phenolic compounds, followed by addition of the excess of derivatizing agent and holded at a temperature of 50-80°C. Scientists [12] studied

 Printed in Kazakhstan

and optimized derivatization by variating temperature from 50 to 80°C and time from 10 min to 24 hrs. It was found that, mixture should be holded at 70°C for 2 hours for the completion of the reaction. Derivatization reactions on the example of interaction quercetin with BSTFA:



Determination of flavonoids may be carried out with using the gas chromatography method, which is one of the most sensitive methods of analysis. For identification of flavonoids with gas chromatography using flame ionization and mass spectrometric detectors.

Thus, gas chromatography with mass spectrometric detector becamewidespread receives for determination and identification phenolic compounds in plant samples, because it ensures component separation of complex natural matrices. In this article we solved the problem of flavonoids identification in plant samples by gas chromatography-mass spectrometry with pre derivatization. Analysis by GC- MS was carried out on a chromatograph Agilent 7890A / 5975C (USA). For automation of selection, sample preparation and sample introduction, gas chromatography-mass spectrometer equipped with an autosampler CTC-Combi-PAL (CTC Analytics AG, Switzerland). To control the whole gas chromatographic system, recording and processing of chromatographic data using software Agilent MSD ChemStation (version 1701EA). Processing included the determination of the retention times, peak heights and areas as well as processing of resulting in a mass spectrometric detector spectral information. To decrypt the obtained results was used the mass spectra library Wiley 7th edition and NIST'02 (the total number of spectra in the library - more than 550 thousand.). Chromatographic parameters are presented in Table 1.

Materials and methods

Auxiliary materials for GC -MS

Helium grade "A" (99.995%) (Orenburg, Russia);

Column: DB-5MS, length 60 m, internal diameter 0.25 mm, film thickness 0.25 μm (Agilent, USA);

Microsyringe for Combi-PAL autosampler, volume of 10μL (Hamilton, Switzerland);

Conical vials 1.5 mL with teflon caps and ultrapure silicone gaskets (CTC Analytics AG);

For derivatization, 50μL of individual flavonoids were dried and placed vials. 50 μLof N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) and 50 μL of pyridine were added and the mixture was kept for 2 hours on water bath at 70°C and then chromatographed.

Analysis by GC- MS was carried out using Agilent 7890A/5975C (USA). For automation of sample preparation and introduction gas chromatography-mass spectrometer is equipped with CTC-Combi-PAL (CTC Analytics AG, Switzerland). Agilent MSD ChemStation (version 1701EA) software was used to control the whole gas chromatographic system, to recording and to process chromatographic data. Processing included determination of retention times, peak heights and areas as well as processing of resulting mass spectral information. Wiley 7th edition and NIST'02mass spectra library (the total number of spectra in the library - more than 550 thousand.) was used to process the obtained results was used Chromatographic parameters are presented in Table 1.

Results and discussion

Objects of study are eight species of Artemisia wormwood:

- Artemisia juncea Kar.et Kir;
- Artemisia terrae-albaeKrasch;
- Artemisia scopariaWaldst. et Kit;
- Artemisia dracunculus L;
- Artemisia sieversianaWilld;
- Artemisia nitrosaWeb.exStrechm;
- Artemisia vulgaris L;
- Artemisia Absinthium L.

Flavonoids are used as standard samples:

Quercetin 95%; Myricetin 96.0%; Kaempferol 97.0%; Naringenin 95%; Hesperetin 95%; Apigenin 97%; Catechin 99.0%; Isorhamnetin95.0%; Luteolin97.0% (Sigma Aldrich) and Ruthin 96.18% (Russian Federation).

Solvents:

– Distilled water;

– Concentrated hydrochloric acid (Russian Federation);

– Methanol (AppliChem);

– Chloroform 99.8% (Sigma Aldrich).

Eluents:

– Acetonitrile 99.8%, (Sigma Aldrich);

– Analytical grade phosphoric acid (Reahim, Russia).

For determining flavonoids, plant samples were kept and preparated according to the scheme shown on Figure 1.

Table 1 – Chromatographic parameters of flavonoids by GC-MS

Parameter	Value
Inlet temperature	240°C
Flow rate carrier gas (helium)	1 mL / min (constant flow)
Oven temperatureprogramme	40 ° C (holding 10 min), heating to 300°C at the rate of 10°C for min (20 min)
MSD interface temperature	240°C
Detection mode	m/z 34-1000 SCPN
Analysis time, min	56

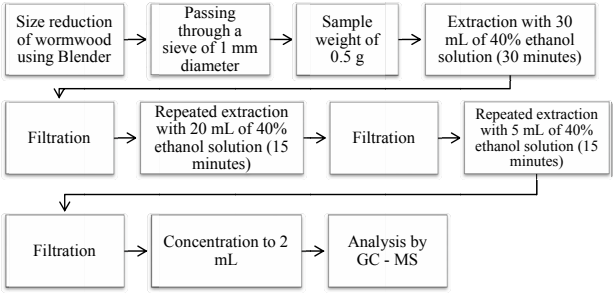
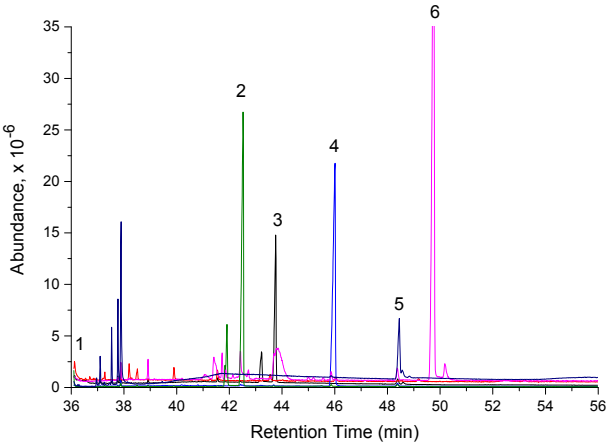


Figure 1 – Scheme of sample preparation of plants to analyze



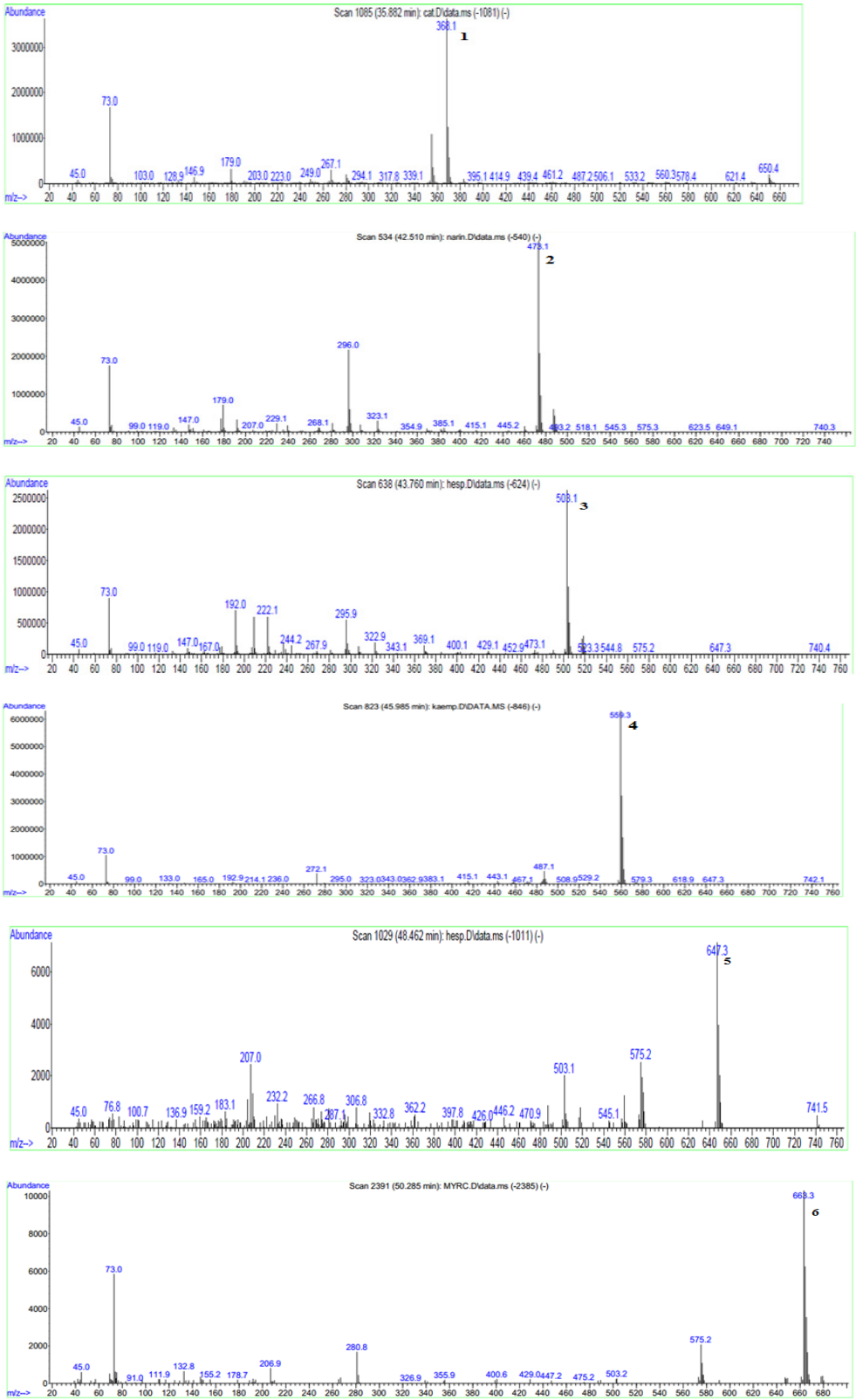
1 – catechin; 2 – naringenin; 3 – hesperidin; 4 – kaempferol; 5 – quercetin; 6 – myricetin.

Figure 2 – Chromatogram of the individual flavonoids

As a result of carried research separation of mixed flavonoids is achieved. Chromatogram of separation six standard flavonoids is presented below on Figure 2.

On the Figure 3 trimethylsilyl (TMS) derivates of six flavonoids are shown according to their eluation order.

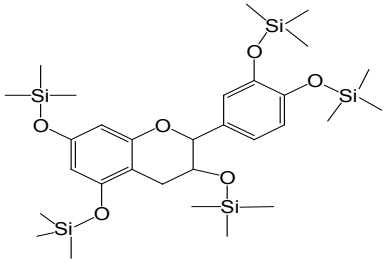
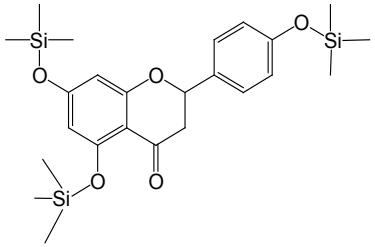
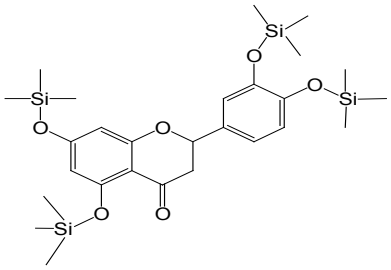
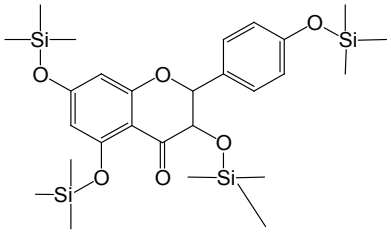
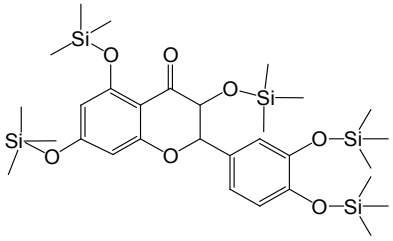
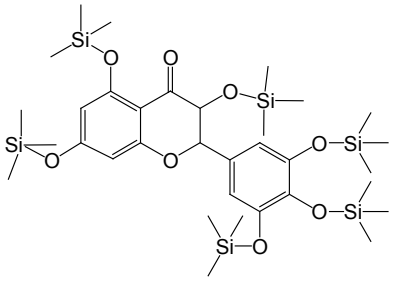
For flavonoids identification parameters of retention and retention time of specific ions and their TMS-derivates are set (Table 2). According to the mass spectra of TMS derivatives various fragment ions compoundsare formed in the result of ionization/



1 – catechin; 2 – naringenin; 3 – hesperidin; 4 – kaempferol; 5 – quercetin; 6 – myricetin.

Figure 3 – Mass spectra of individual flavonoids

Table 2 - Parameters of identification of flavonoids

Compound	Formula of TMS derivatives	MM	Number of OH groups	Retention time, min	m / z
Catechin		650	5	35.8	368 (999) 369 (338) 355 (295) 370 (157)
Naringenin		488	3	42.5	473 (847) 296 (458) 474 (334) 179 (234) 475 (154) 297 (125) 177 (123)
Hesperidin		519	3	43.7	503 (999) 504 (421) 192 (295) 222 (217) 209 (215)
Kaempferol		577	4	45.9	559 (999) 560 (489) 487 (65)
Quercetin		663	5	48.4	647 (999) 648 (567) 649 (330) 559 (169) 650 (112)
Myricetin		751	6	50.2	735 (452) 736 (285) 737 (180) 647 (143)

Identification of flavonoids was performed by retention parameters and mass spectra of substances. From all the samples selected in 2013, flavonoids were found only in sample №3, such as kaempferol and quercetin. The chromatogram of the wormwood extracts №3 - Artemisia scopariaWaldst. et Kit is presented in Figure 4.

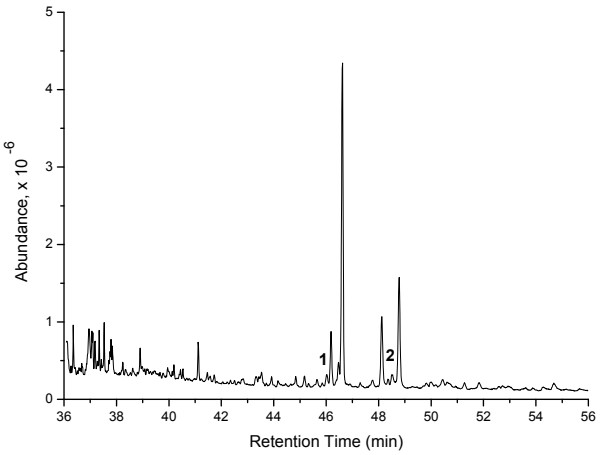


Figure 4 – The chromatogram of the wormwood extracts №3 – Artemisia scopariaWaldst. et Kit
(1 kaempferol – 45.9 min 2 – quercetin – 48.4 min)

Conclusion

According to the analysis of samples of wormwood were summed the following conclusions:

1. Before testing on GC- MS is necessary to remove of flavonoids aglycones and polar solvents in the sample because the sugar overload operation of the mass spectrometer;

2. Application derivatizing agent contributes to the volatility of investigated substances, thermal stability and better separation;

3. In the sample of wormwood Artemisia scopariaWaldst. et Kit were found kaempferol and quercetin, which are then assumed to allocate from this type of plant.

References

1. Zhang Q, Ye M.(2009) J. Chromatogr. A.1216:1954-1969

2. LinderborgK., LaaksonenA., KallioH., YangB. (2011) Food Res. Int. 44:2027-2033

3. ZhengJ., YangB., TuomasjukkaS., OuSh., KallioH.(2009) J. Agric. Food Chem57:2977-2987

4. Creaser C., Koupai-Abyazani M., Stephenson G.(1989) J. Chromatogrol. 478:415-421

5. Fenner G. (1996) J. Agric. Food Chem. 44:3727-3729.

6. ZhangK., Zuo Y.(2004) J. Agric. Food Chem. 52:222-227.

7. AnliErtan, VuralN., Kizilet E.(2008) JI Brewing.114:239-245

8. Garcia-VillalbaR., PacchiarottaT., Carrasco-PancorboA., Segura-CarreteroA., Fernandez-GutierrezA., DeciderA., MayborodaO.(2011) J.Chromatogr.1218:959-971.

9. Loots D., van der WesthuizenF., BotesL. (2007) J. Agric. Food Chem. 55:6891-6896.

10. Soleas G., Diamandis E., Karumanchiri A., Goldberg D.(1997) Anal. Chem. 69:4405-4409.

11. Horvat R., Senter S.(1980) J. Agric. Foal Chem. 28:1292-1295.

12. Razborsek M., Voncina D., DolecekV., Voncina E.(2007) ActaChim. Slov. 54:60-67.

13. Kashutin M., Ioffe S., Tartakovsky V.(1975) Usp9:1620-1648.