P19: SIMULTANEOUS DETERMINATION OF *α*- THUJONE, *β*-THUJONE AND CAMPHOR IN INFUSION OF SAGE USING ATTENUATED TOTAL REFLECTANCE FOURIER TRANSFORM INFRARED SPECTROSCOPY.

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Abstract – Determination of *α*-, *β*-thujone and camphor in sage infusions was achieved using attenuated total reflectance infrared Fourier transform infrared spectroscopy (ATR-FTIR) (spectral region 1801-1408 cm^{-1} in 2nd derivative form) and partial least square method (PLS) . The correlation coefficients (R^2) and the root-mean-square error of calibration (RMSEC) of *α*-, *β*-thujone and camphor were 0.99, 0.0210, 0.96, 0.0180 and 0.99, 0.0697, respectively. The proposed method is simple, rapid and economical.

Keywords: thujone, camphor, infusion, sage, ATR-FTIR.

1. INTRODUCTION

Salvia sp. (sage) is a medicinal and aromatic plant that is consumed mainly as infusion in Greece [1]. Due to its beneficial health properties sage infusions have attracted scientists and consumers attention. The compounds *α*-, *β*- thujone and camphor are contained in various preparations of sage. According to various agencies (EFSA) and clinical studies, *α*-, *β*- thujone and camphor show toxic effects in humans over established limits [2], [3]. Sage infusions were studied to determine the concentrations of these components due to their high content in them.

Analytical techniques such as gas chromatography coupled with mass spectrometry (GC-MS) is the usual tool to determinate volatile components in herbal extracts however they are time-consuming and expensive. With other techniques like attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR), the determination is also efficient, effective and faster [4].

The aim of this study was to investigate the use of ATR-FTIR spectroscopy for the determination of above compounds in sage infusions.

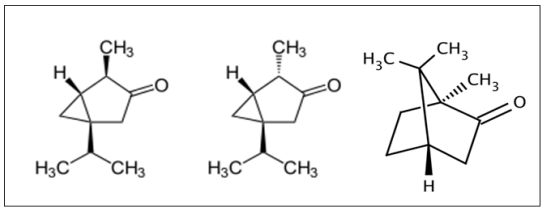

Figure 1. Chemical structure of *α*-, *β*-thujone and camphor.

2. EXPERIMENTAL

Dried leaves of seven different sages were obtained from several areas around Greece by local growers (Table 1). The different species of sage were native of each region. Each plant material was powdered in an electrical blender.

Sage infusions were prepared by stepping 2 g of dry powdered leaves in 200 mL of boiled water (~90°C) for 10 minutes, followed by filtration. This procedure simulates the conventional procedure of tea preparation. The resulted sage infusions were extracted with organic solvent (diethyl ether), in order to isolate the volatile compounds, *α*-, *β*-thujone and camphor. Before the analysis each organic extract of infusion was concentrated under nitrogen steam.

Volatile content of organic extracts of each infusion was determined using gas chromatography (Thermo Scientific, TRACE GC ULTRA) coupled with mass spectrometry (DSQ II). Thujone (*α*- and *β*-) and camphor were identified based on retention times, spectroscopic data of standard compounds and literature data (Adams 2007). Calibration curves of each compound and method of internal

standard (cyclohexanone) were used for quantitative determination.

Table 1. Origin of *Salvia* species.

Species	Region	Sample Code
S.triloba	Western Greece (Ilia)	ST
S.pomifera	West Greece (Agrinio)	SP
S.sclarea	South Aegean (Kalymnos)	SS
S.fruticosa	Epirus (Syvota)	SF
S.officinalis	Central Greece (Aetolia-Acarnania)	SOF1
S.officinalis	Central Macedonia (Thessaloniki)	SOF2
S.officinalis	West Macedonia (Kozani)	SOF3

Spectra of condensed organic extracts of sage infusions and pure standards were collected with a Thermo Nicolet 6700 FT-IR in the ATR mode (Thermo Fisher Scientific Inc., CA, USA**).** For the simultaneous determination of *α*-, *β*-thujone and camphor calibration curves were obtained using TQ Analyst software (version 8.0.0.245, Thermo Fischer Scientific Inc.) and partial least square (PLS) method. Statistical accuracy of calibration model was described by the correlation coefficient (R^2) and the root-mean-square error of calibration (RMSEC). The 2nd derivative spectra of the 1801 to 1408 cm^{-1} of each averaged spectrum and the corresponding concentrations (actual values) were used for the PLS method.

3. RESULTS AND DISCUSSION

Quantification of *α*-, *β*-thujone and camphor in sage infusions is important due to existed limits in the consumption of food and drinks that contain sage. Results of quantitative determination of the above compounds using GC-MS are shown in Table 2. Concentration levels of *α*-thujone estimated from 0.002 mg/mL to 0.521 mg/mL. The quantities of *β*-thujone in sage infusions ranged from 0.002 mg/mL to 0.194 mg/mL. For camphor the corresponding quantities ranged from 0.172 mg/mL to 1.284 mg/mL. In all samples *α*-thujone predominated among two diastreomeric forms. Regarding camphor, sample ST presented the highest concentration and at sample SF was not found. The highest concentration of thujone (*α*- and *β*-) was detected at sample SP, while sample SS showed the lowest. Concentration levels of above compounds at sage infusions varied due to each region native species.

Table 2. *α*-, *β*-thujone and camphor content (mg/mL) in sage infusions from different origin.

Sample	α-thujone (mg/mL of sage infusion)	β-thujone (mg/mL of sage infusion)	camphor (mg/mL of sage infusion)
ST	0.022	0.012	1.248
SP	0.521	0.194	0.558
SS	0.007	0.010	0.418
SF	0.002	0.002	0.000
SOF1	0.258	0.034	1.104
SOF2	0.012	0.006	0.172
SOF3	0.139	0.035	0.525

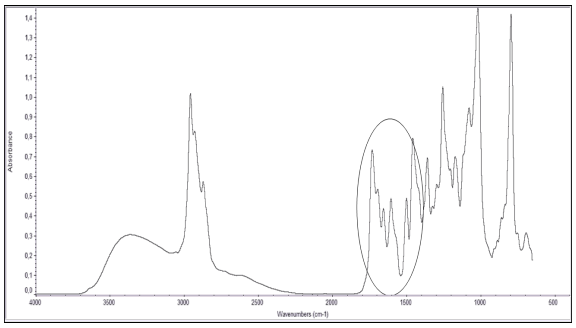

Figure 2. Typical ATR-FTIR spectra of organic extract of *Salvia* sp. infusion.

Fig.2 shows a typical ATR-FTIR spectrum of organic extract of *Salvia* sp. infusion. All spectra had

similar profile and proportional to the quantities of the components. For the determination of *α*-, *β*-thujone and camphor the correlation coefficient was calculated 0.99, 0.96 and 0.99, respectively. The corresponding root-mean-square error of calibration (RMSEC) was found 0.0210, 0.0180 and 0.0697 (Fig.3). The results were compared with those obtained from GC-MS analysis.

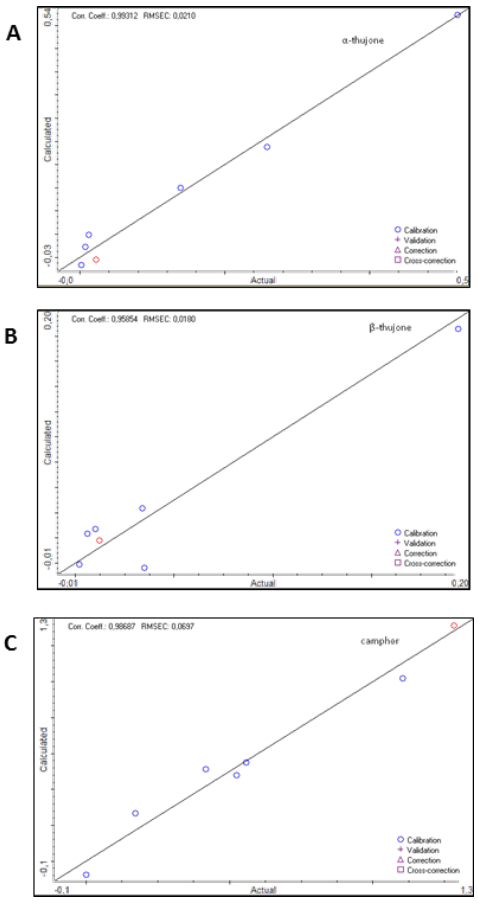

Figure 3. Linear correlation between the reference and the calculated values and determination of R^2 and RMSEC of *α*-thujone (A), *β*-thujone (B) and camphor (C) according to the ATR technique.

The proposed ATR method according to above corresponding values showed a linear relationship between the reference concentrations of *α*-, *β*-thujone and camphor and calculated ones, therefore this method is a robust and reliable method for determination of these three compounds in sage infusion.

4. CONCLUSIONS

In this study was developed a simple, rapid and cost-effective analytical method for the determination of *α*-, *β*-thujone and camphor in sage infusions using ATR-FTIR.

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