

P 17: ATR-FTIR Spectroscopy on intact dried leaves of sage (*Salvia officinalis* L.) – chemotaxonomic discrimination and essential oil composition



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Abstract

Sage (*Salvia officinalis* L.) is cultivated worldwide for its aromatic leaves which are used as herbal spice and for phytopharmaceutical applications. Fast analytical strategies for essential oil analysis, performed directly on plant material would reduce the delay between sampling and analytical results. This would enhance product quality by improving technical control of cultivation. The attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy method described here provides a reliable calibration model for quantification of essential oil components (EOC) and its main constituents (e.g. α -thujone and β -thujone) directly on dried, intact leaves of sage. Except for drying no further sample preparation is required for ATR-FTIR and the measurement time of less than 5 min per sample contrasts with the most common alternative of hydro-distillation followed by GC analysis which can take several hours per sample.

Keywords: infrared spectroscopy, essential oil, thujone, quantification, PLS, nondestructive, ATR-FTIR, *Salvia officinalis* L., chemometry

Introduction

Sage (*Salvia officinalis* L.) is a popular aromatic plant found on all continents except Antarctica and Australia (AL-QUDAH et al., 2014; BEN FARHAT et al., 2009; GRAUSGRUBER-GRÖGER et al., 2012; LAMIEN-MEDA et al., 2010). The pharmaceutical uses and aromatic taste of sage are largely derived from the essential oil that is rich in thujone and camphor with α - and β -pinene, 1,8-cineole or camphene as minor constituents.

Today a large number of genetically different accessions of *S. officinalis* L. have been described that are characterized by varying content of essential oil and high diversity of the related chemical composition (LAMIEN-MEDA et al., 2010). Furthermore, content and composition of essential oil depend on climatic and cultivation conditions, on developmental stage of the plant/leaf, origin of plant material and even on daytime of harvest. Hence, for both, plant breeding and cultivation, the exact content and composition of the essential oil is needed to ensure that the final products are of consistent and high quality (PHARMACOPOEIA, 2014).

Traditional analyses of essential oil based on gas chromatography (GC) are time-consuming and expensive and also require laboratory infrastructure as well as specially trained personnel. Hence, analytical methods directly applicable to intact plant material which are more efficient, faster and robust are demanded. With the ATR-FTIR technique, mono-layers of analytes can be investigated through direct contact between sample and the IR-crystal. Since the essential oil in *S. officinalis* L. is located in special glandular trichomes on the leaf surface, ATR-FTIR offers a promising alternative to GC.

Therefore, the aim of this study was to investigate the use of ATR-FTIR spectroscopy for the direct qualitative and quantitative analysis of the essential oil composition of dried intact leaves of sage and including the effect of different varieties, leaf age and date of harvest. Based on reference analysis of solvent extracts from the plant material by GC appropriate calibration models for es-

essential oil content and composition can be developed that include the chemical diversity of different sage accessions.

Materials and Methods

Seeds of 12 different sage (*Salvia officinalis* L.) accessions were obtained from the Leibniz Institute for Plant Genetics and Crop Plant Research (IPK, Gatersleben, Germany). Seeds of one additional commercial sage accession were obtained from Bingenheimer Saatgut GmbH (Echzell, Germany). Plants were cultivated at the experimental field of the Julius Kühn-Institute in Berlin (Germany) and leaf samples were taken for different developmental stages and at two different dates of harvest.

The ATR-FTIR spectra of intact dried sage leaves and pure standards were recorded with a portable ATR diamond crystal infrared spectrometer (Alpha, Bruker Optics GmbH, Ettlingen, Germany).

For estimating the variability of the samples, principal component analysis (PCA) was used based on the instrument software package OPUS 7.2 (Quant 2 module, Bruker Optik GmbH, Germany) and Unscrambler X (Camo, Norway).

Detailed botanical description, experimental information for solvent extraction, GC-FID analysis, infrared spectroscopic measurements and statistics are given in the appropriate literature (GUDI *et al.*, 2015).

Results

Sage is known to show a large variety in essential oil content and composition (GRAUSGRUBER-GRÖGER *et al.*, 2012; LAMIEN-MEDA *et al.*, 2010). Information about chemical heterogeneity of the accessions is highly relevant for the development of suitable calibration models with FTIR-ATR spectroscopy (where only a small amount of material is used with each measurement) since it determines an appropriate sampling and statistical evaluation.

Therefore, a representative number of plants (repetitions per accession) has to be incorporated and a greater degree of averaging ATR-FTIR spectra (summarizing variability by reduction of data points) has to be applied.

The use of spectra averaged across accessions leads to an improved prediction not only for the EOC yield and major constituents (α -thujone, β -thujone, camphor) but also for components with lower concentrations (in per mill range) such as α - or β -pinene and 1,8-cineole.

For all essential oil components, coefficients of determination (R^2) higher than 0.86 were obtained with low values for BIAS and RMSECV of less than 10 % of the corresponding mean values (GUDI *et al.*, 2015).

Furthermore, the robustness of the validated models was investigated with an additional test-set validation of 12 independent samples (3 sets of 10 plants, old/young, H1/H2) which came from the commercial sage accession.

As shown by the values of R^2 , RMSEP and BIAS given in Table 1, for essential oil components, a robust and reliable prediction model could be achieved with $R^2 = 0.98$. The relatively low prediction accuracy for individual metabolites could be explained by the genetic and chemotaxonomic diversity of the investigated sage accessions.

Tab.1 Statistical parameters for test-set validation of the developed prediction models of plant wise averaged spectra (n=48) with 12 independent samples from the commercial accession.

component	R ²	RMSEP*	BIAS*	Range*/mean*	LV**
EOC content	0.98	0.174	0.0371	2.223 - 5.514 / 3.869	7
α -thujone	0.74	0.124	0.061	0.547 - 1.268 / 0.908	8
β -thujone	0.80	0.0778	0.0375	0.085 - 0.593 / 0.339	5
camphor	0.74	0.09	-0.0768	0.253 - 0.895 / 0.574	7
1,8-cineole	0.87	0.0482	0.0241	0.142 - 0.598 / 0.37	6
α -pinene	0.55	0.0471	0.0184	0.056 - 0.312 / 0.184	7
β -pinene	0.84	0.0111	0.00267	0.02 - 0.103 / 0.0615	8

ml 100 g⁻¹ DM, ** Number of Latent Variables (LV)

In the current ATR-FTIR quantification protocol plant material only needs to be dried and spectral measurements take only a few minutes. This allows rapid quality control of the material when it is first received for processing compared to the time-consuming analysis recommended in the Pharmacopoeia.

Transfer of the current analytical protocol to fresh material and application in the field is currently under investigation. This will undoubtedly improve growing and quality control for both plant breeding and cultivation and assist in evaluating the chemical variation of breeding populations and identifying optimal points for harvest.

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