

# Authenticity Control of Alpine Aromatic Plants by Enantioselective Gas Chromatography–Mass Spectrometry

## Part 1: White genepi (*Artemisia umbelliformis* Lam.)

Matthias Wüst\* and Umberto Piantini

**Abstract:** A method for the authenticity control of the alpine aromatic plant white genepi (*Artemisia umbelliformis* Lam.) was developed by means of coupling headspace solid-phase microextraction with enantioselective GC–MS. By using heptakis[2,3-di-O-methyl-6-O-tert.-butyldimethylsilyl]- $\beta$ -cyclodextrin as stationary phase the chiral monoterpenes borneol,  $\alpha$ -pinene,  $\beta$ -pinene, and  $\alpha$ -terpineol could be analyzed simultaneously allowing their enantiomeric ratios to be used as reliable indicators of genuine white genepi plant material.

**Keywords:** *Artemisia umbelliformis* · Authenticity control · Enantioselective GC–MS · Headspace solid-phase microextraction · White genepi

### Introduction

The market for natural and organic-grown products is growing continuously. Therefore, more than 15 years ago, research on medicinal and aromatic plants and their production started in Switzerland. Nowadays more than 45 species are cultivated on an overall surface of 300 hectares, producing some 300 tons of dried plants [1]. Aiming at a diversification of alpine crops in particular, precedent work on the domestication and breeding of white genepi (*Artemisia umbelliformis* Lam.) has been published [2]. This plant grows between an altitude of 2000 and 3200 meters and is traditionally used for the preparation of liqueurs, 'eaux de vie', and digestive teas. However, the domesticated plant can be cultivated between 1000 and 1600 meters. Its essential oil contains mainly chiral

monocyclic and bicyclic monoterpenes and sesquiterpene lactones [3][4]. In view of chemotaxonomic studies genuine enantiomeric ratios of these chiral terpenes are of special interest. Moreover, the determination of enantiomeric ratios has been demonstrated as an outstanding method in the authenticity control of genuine chiral compounds and is used in the quality assurance of natural flavors and essential oils [5].

To achieve an efficient quality control of white genepi and to protect this valuable natural raw material against adulteration we have therefore developed an enantioselective gas chromatographic method that allows the simultaneous stereodifferentiation of several chiral monoterpenes.

### Results

The simultaneous stereodifferentiation of the chiral monoterpenes  $\alpha$ -pinene (**1**),  $\beta$ -pinene (**2**), borneol (**3**), and  $\alpha$ -terpineol (**4**) was achieved using heptakis[2,3-di-O-methyl-6-O-tert.-butyldimethylsilyl]- $\beta$ -cyclodextrin, which has been shown to be a highly versatile chiral stationary phase by

Mosandl and coworkers [6]. The Fig. (see a) shows the separation of a standard mixture. All enantiomeric pairs show resolutions of at least 1.5 allowing an accurate determination of the corresponding enantiomeric ratios.

For isolation of the essential oil, rapid headspace solid-phase microextraction (HS-SPME) of the herb was chosen, allowing us to skip a time-consuming steam distillation step. The Fig. (see b) shows a typical chromatogram obtained by this method. All target compounds are well resolved and their enantiomeric ratios can be determined accurately without any interference owing to the less complex composition of the genepi essential oil. However, it is noteworthy that for the analysis of more complex essential oils or trace components multidimensional chromatographic techniques are highly recommended to avoid peak interferences, which may occur even in SIM-detection mode when using a mass selective detector [5].

The Table summarizes the results that were obtained for several white genepi plants originating from different locations in Switzerland, France, and Italy.

\*Correspondence: Prof. M. Wüst  
University of Applied Sciences Valais  
Department of Life Technologies  
Route du Rawyl 47  
CH-1950 Sion 2  
Tel.: +41 27 606 8654  
Fax: +41 27 606 8515  
E-Mail: matthias.wuest@hevs.ch

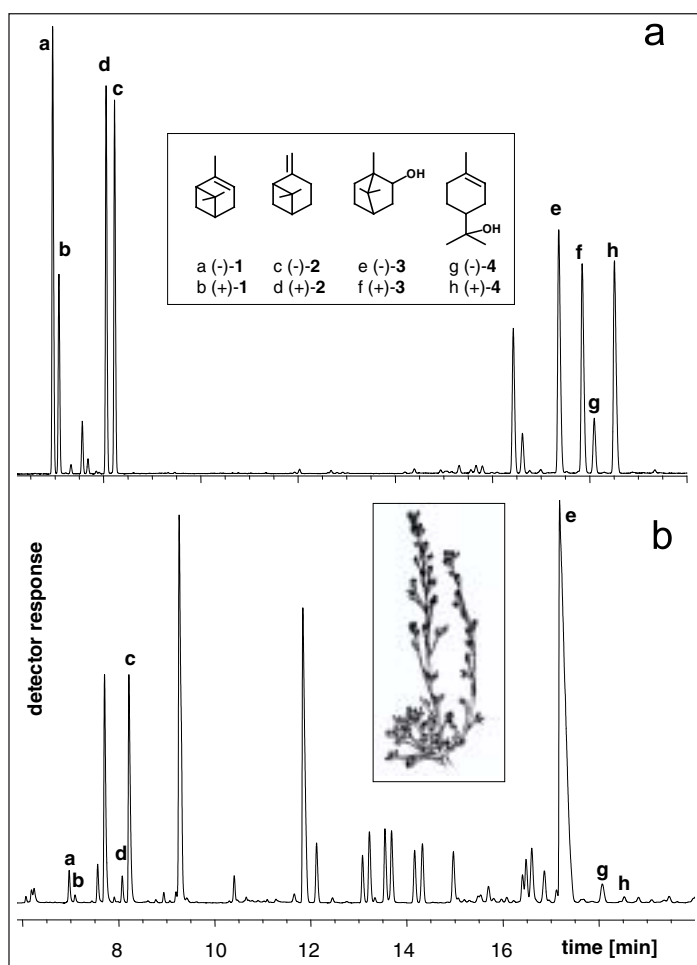


Fig. a) Enantioselective analysis of a standard mixture of  $\alpha$ -pinene (**1**),  $\beta$ -pinene (**2**), borneol (**3**), and  $\alpha$ -terpineol (**4**); b) enantioselective analysis of a HS-SPME sample from *A. umbelliformis*

## Discussion

The aim of this study was to establish authenticity parameters that allow the quality control of white genepi by means of HS-SPME/enantioselective GC-MS. From the data given in the Table it can be concluded that the enantiomeric purities of  $\alpha$ -pinene (**1**),  $\beta$ -pinene (**2**), borneol (**3**), and  $\alpha$ -terpineol (**4**) are reliable indicators of genuine white genepi plant material. (1*S*)-(-)-Borneol ((-)-**3**) is present in high enantiomeric purities (>99%), independent from the origin of the plant. All other monoterpenes analyzed show some natural variability with respect to their enantiomeric purity. This rather surprising result is in contrast to the widespread opinion that nature produces only enantiomerically pure compounds as the result of the enzyme-catalyzed biosynthesis. However, recent publications in this field have given sound evidence that several plants are able to biosynthesize both enantiomers of a chiral compound, because two different enzymes with opposing stereospecificities are present *in planta*. This has been demonstrated very recently by Croteau and coworkers cloning (+)- and

(-)- $\alpha$ -pinene synthases from loblolly pine (*Pinus taeda*) [7]. The enantiomeric ratios are consequently determined by the activity ratios of both enzymes and may be influenced by external parameters acting on plant physiology and metabolism. This natural variability has to be kept in mind when enantioselective GC is applied in purity control of essential oils [8]. However, as far as the enantiomeric ratios of  $\alpha$ -pinene (**1**),  $\beta$ -pinene (**2**), and  $\alpha$ -terpineol (**4**) in white genepi are concerned, it is possible to define limits for their values as authenticity parameters:  $\alpha$ -Pinene (**1**) shows enantiomeric purities better than 70% in favor of the (1*S*)-enantiomer (-)-**1**,  $\beta$ -pinene (**2**) shows enantiomeric purities better than or equal to 90% in favor of the (1*S*)-enantiomer (-)-**2**, and  $\alpha$ -terpineol (**4**) shows enantiomeric purities better than 65% in favor of the (4*S*)-enantiomer (-)-**4**. Any deviation from these authenticity parameters may be interpreted as a falsification by addition of non-authentic plant material or synthetic racemates on a preliminary base. Due to the natural variability of the enantiomeric ratios the establishment of a more comprehensive database, which includes

Table. Enantiomeric ratios of  $\alpha$ -pinene (**1**),  $\beta$ -pinene (**2**), borneol (**3**), and  $\alpha$ -terpineol (**4**) from *A. umbelliformis* from different locations. The enantiomeric ratios are expressed in percent of the predominant enantiomer. For each location two samples were analysed. For each sample the mean of at least two determinations is given.

Origin	$\alpha$ -Pinene (-)- <b>1</b>	$\beta$ -Pinene (-)- <b>2</b>	Borneol (-)- <b>3</b>	$\alpha$ -Terpineol (-)- <b>4</b>
Fenis	73	90	>99	74
	74	94	>99	73
Liddes	91	96	>99	88
	89	96	>99	71
Leschaux	78	94	>99	82
	83	93	>99	79
Châtillon	83	92	>99	78
	73	91	>99	69

authentic data collected over a period of several years is mandatory. Our analytical method and data presented in this paper is an essential prelude to reach this aim.

## Experimental

Flowering white genepi plants from locations as indicated in the Table were collected during 2000/2001. Approximately 1 g of homogenized dried plant material (whole aerial parts: stems, leaves, twigs, flowers) was placed in a 20 ml vial for equilibration (30 min) following head-space sampling by SPME [9] (SUPELCO polyacrylate fiber, 85  $\mu$ m). Enantioselective GC-MS and GC-FID analysis was carried out using heptakis[2,3-di-O-methyl-6-O-tert.-butyldimethylsilyl]- $\beta$ -cyclodextrin in SE-52 as chiral stationary phase [6].

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