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SUPERCRITICAL FLUID EXTRACTION OF VALERIAN ROOT

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Summary

In this study supercritical carbon dioxide extraction from the root of three types of Valerian (Valeriana officinalis L) was investigated. Extractions were performed at temperatures of 313 and 323 K and pressures of 10 and 15 MPa. Effect of particle size on extraction yield was also investigated. The extracts were analyzed by analytical GC (FID) and GC-MS.

Keywords: *Valerian; Supercritical fluid extraction; Bornyl acetate; Valerenal; Isovaleric acid*

Introduction

Preparations on the basis of extracts of Valerian root (*Valeriana officinalis*, Valerianaceae) are used as mild sedatives for reduction of tension, nervousness and irritation, to help better dream and to prevent agitated sleep. In modern phytotherapy, this usage is based on the experience of traditional medicine (the root of valerian is used more then 2000 years in this purpose), as well as on the results of chemical, pharmacological and numerous clinical studies. The oil and absolute are also used as fragrance components in soaps and in moss and forest fragrances. It is used to flavor tobacco, root beer, liquors and apple flavorings [1]. Dried roots of valerian are sometimes packed in cloth bags and put around grain, fruits, berries, or vegetables, to fend off insects, rodents, and other damaging animals [2].

Drug *Valerianae radix* consists of dried underground parts according to European Pharmacopoeia should contain not less than 5 ml/kg of essential oil for the whole drug and not less than 3 ml/kg of essential oil for the cut drug, both calculated with reference to the dried drug and not less than 0,17 % of sesquiterpene acids expressed as valerenic acid. The essential oil of the Valerian root contains monoterpenes and sesquiterpenes, and after longer distillation low volatile sesquiterpenoides (valerenic, hydroxyvalerenic and acetoxyvalerenic acid). Fresh drug contains valepotriates, which are very unstable. Degradation of valepotriates leads to the formation of unsaturated iridoide aldehydes and corresponding acids, from which isovaleric one is responsible for specific unpleasant smell of older drug. Valerian root contains alkaloids and amino acids. In spite of many studies, mechanism of action of the extract of Valerian root is not completely explained. Activity is result of synergistic action of greater number of components [3-5]. Consequently, nowadays, valerian as one among 10 top-selling herbal supplements is considered to be highly respected medical plant species.

Hydrodistillation is a wide-spread method for production of essential oil of Valerian in industry and it has been studied extensively as well. Evan if the simplicity and low investment and operative costs of this method are taken into account, the fact that some of the high volatile and hydro soluble substances will be forever lost is undeniable. Alcohol extraction is often used for isolation of active components in order to preserve thermo labile and highly volatile compounds, but it requires organic solvent use. Moreover, severe legislative restrictions are currently being proposed to eliminate solvent residues in these products when used in the food, pharmaceutical and cosmetic industries. Some of these problems can be solved by employing supercritical fluid in essential oil extraction [6]. Although chemical composition of valerian oil obtain by hydrodistillation was studied

extensively, a little work has been published about isolation of active substances by supercritical fluid extraction (SFE) from valerian drug. This paper concerns with pressure, temperature and pretreatment influence on SFE yield, as well as with chemical composition of supercritical extracts.

Material and methods

Three sorts of valerian (*V. officinalis* L.) were analyzed: wild grown valerian from eastern Serbia (valerian I), wild grown valerian from central Serbia (valerian II) and cultivated sort (Arterner züctung) grown in northern Serbia (valerian III).

Extractions with SC CO_2 were carried out in the Autoclave Engineers Screening System shown in Fig. 1. The Supercritical Extraction Screening System is designed for small batch research runs using CO_2 as the supercritical medium with maximum allowable working pressure of 41.3 MPa at 511 K. Liquid CO_2 is supplied from CO_2 cylinder by a siphon tube. The CO_2 is pumped into the system by the liquid metering pump untill the required pressure is obtained. Back preassure regulators are used to set the system pressure (in extractor and separator). The extractor vessel (150 ml) is filled with the plant material from which a substance is to be extracted. Heaters are supplied on the extractor vessel for temperature elevation. The SC CO_2 flows through the extractor and enters the separator vessel. Samples of the extracted substance can be taken by opening the ball valve located at the bottom of the vessel. A flowmeter is provided to indicate the flow rate of CO_2 being passed through the system and the flow can be adjusted by micrometering valve. The CO_2 continues to flow out of the separator through the flowmeter/totalizer and out to atmosphere. Valerian root was fine milled and sieved to three fractions with average diameters: 0.4, 0.65 and 0.7 mm. Mass of the plant sample was 47g and the solvent flow rate was 0.3 kg/h in all experiments. Extractions were carried out at temperatures of 313 and 323 K and pressures of 10 and 15 MPa. The influence of particle size on SFE was investigated in the case of Valerian I, the influence of extraction conditions (T,P) was investigated in the case of Valerian II, and the SFE from the cultivated sort was performed at 313 K and 10 MPa.

Qualitative and quantutative analyses of the SFE extracts were carried out using a Hewlett-Packard GC (FID) and GC-MSD analytical systems. Model HP-5890 Series II, equipped with a split-splitless injector, HP-5 capillary column (25 m · 0.32 mm, film thickness 0.52 μm) and a flame ionization detector (FID), was employed. Hydrogen was used as carrier gas (1 ml/min). The injector was heated at 250°C, the detector at 300°C, while the column temperature was linearly programmed from 40-260°C (4°/min). GC-MSD analyses were carried out under the same analytical conditions. Model HP G 1800C Series II GCD analytical system equipped with HP-5MS column (30 m · 0.25 mm · 0.25 μm) was used. Helium was used as carrier gas. The transfer line (MSD) was heated at 260°C. The EIMS spectra (70 eV) was acquired in the scan mode in the m/z range 40-400. In each case, sample solution in hexane (1 μl) was injected in split mode (1:30).The identification of constituents was performed by matching their mass spectra and Kováts indices (I_K) with those obtained from authentic samples and/or NIST/Wiley spectra libraries, different types of search (PBM/NIST/AMDIS) and available literature data (Adams). Flame ionization detection area %, obtained by the integration of corresponding chromatograms, was used for quantification of individual components.

Storage tank

pump

Fig. 1. Schematic presentation of The Autoclave Engineers Screening System

Results and discussion

The influence of particle size on extraction yield in the case of SFE from valerian I is presented in Fig. 2. The influence of SFE conditions (T, P) on extraction yield in the case of SFE from valerian II is presented in Fig. 3. SFE extract obtained at 10 MPa and 313 K yielded in the highest quantity of monoterpenes and sesquiterpenes. Therefore, SFE from cultivated sort (valerian III) was carried out at 313 K and 10 MPa with fraction of average particle size of 0.4 mm. Results of the analytical analyses of SFE extracts obtained at 313 K and 10 MPa are presented in Table 1. As can be seen valerenal, bornyl acetate and valerianol are dominant compounds. Supercritical extracts obtained from Valerian III contained much more isovaleric acid than wild grown sorts. Content of bornyl acetate was higher in extracts from Valerian II and III compared to Valerian I. The highest quantity of valerenal was detected in SFE extracts from Valerian III.

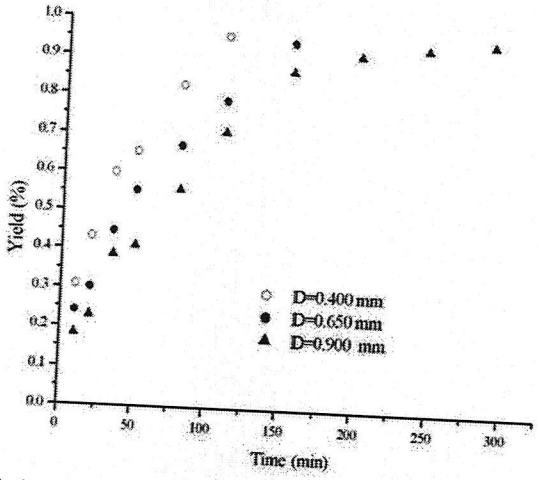

Fig. 2. Effect of particle size on extraction yield in the case of SFE from valerian I at 313 K and 10 MPa

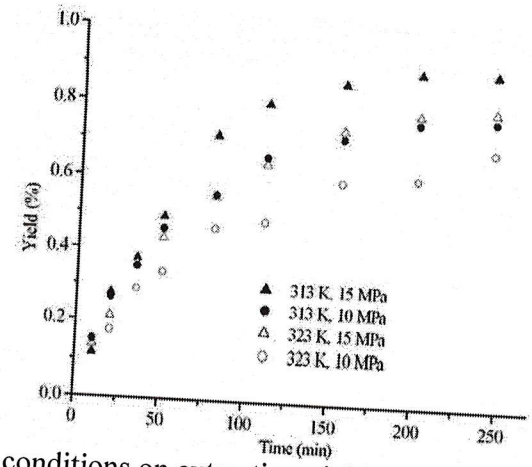

Fig. 3. Effect of SFE conditions on extraction yield in the case of SFE from valerian II

Table 1. Composition of valerian root SFE extract (%w/w) obtained at 313 K and 10 MPa ($I_{K,E}$ experimental values of Kováts indices, $I_{K,A}$ literature values of Kováts indices)

			Valerian I	Valerian II	Valerian III
	$I_{K,E}$	$I_{K,A}$	%	%	%
isovaleric acid	901,8	836	1,34	5,20	7,48
α-thujene	927,0	930		0,25	0,15
α-fenchene	940,9	953			0,14
p-menthone	1156,6	1153	0,37		0,21
borneol	1163,3	1169	4,80	1,02	0,15
terpinen-4-ol	1175,3	1177			0,19
myrtenol	1195,1	1196	0,30	0,32	0,17
bornyl acetate	1283,1	1289	4,57	7,15	6,86
menthyl acetate	1294,7	1295	0,41		
trans-pinocarvyl acetate	1296,3	1298	0,27		
carvacrol	1303,2	1299	0,37	0,41	2,16
myrtenyl acetate	1323,0	1327	0,34		1,08
δ-elemene	1333,8	1338	0,31	1,47	6,11
α-terpinyl acetate	1347,3	1349		0,22	0,14
pacifigorgia-1(9),10-dien	1383,8	1385	0,92	0,32	0,37
β-cubebene	1385,3	1390	0,27		
β-elemene	1388,0	1391		0,62	0,63
β-longipinene	1403,2	1403	0,27	0,23	0,26
pacifigorgia-1(6),10-diene	1410,2	1415	0,37		0,70
β-caryophyllene	1414,5	1419	0,45	0,64	1,35
2,5-dimetoxy-p-cymene	1421,5	1427	1,61	2,28	1,85
γ-elemene	1429,5	1437	0,38	1,16	1,72
α-humulene	1448,9	1455	0,34		0,49
valerena-4,7(11)-diene	1455,4	1456	2,97	2,06	4,26
γ-gurjunene	1472,2	1477	1,48	0,75	2,17
ar-curcumene	1479,2	1481	1,53	1,42	0,78
β-ionone	1483,3	1489	0,87	1,13	1,48
α-muurolene	1496,0	1500	0,44	1,00	0,44
β-bisabolene	1504,7	1506	0,77	1,46	2,27
bornyl isovalerate	1512,6	1512		0,65	0,16
δ-cadinene	1519,3	1523	0,33	0,27	0,21

kessane	1522,7	1533	1,16	1,06	0,52
pacifigorgiol	1540,4	1539	1,37	0,24	1,13
elemol	1545,7	1550	1,45	0,77	0,76
germacrene B	1554,4	1561	0,98	0,81	0,71
spathulenol	1573,4	1578	4,64	2,53	1,51
caryophyllene oxide	1578,7	1583	0,60	0,88	0,66
globulol	1588,0	1585	0,80	0,67	0,38
eudesm-5-en-11-ol	1589,9		0,46	0,53	0,25
viridiflorol	1597,2	1593		0,37	0,18
cis-α-copaene-8-ol	1624,3		7,93	10,08	2,77
isospathulenol	1633,8	1639	0,46	0,47	0,49
β-eudesmol	1645,0	1651	0,90	1,91	1,69
valerianol	1648,2	1652	2,47	11,29	1,37
α-cadinol	1653,7	1654	0,56	1,91	0,35
valeranone	1667,6	1675	0,44	0,92	0,48
α-bisabolol	1681,4	1686	0,67	0,70	0,26
kessyl alcohol	1690,2		0,32		0,17
valerenal	1715,7	1706	10,69	6,58	12,19
cis-nuciferol	1727,7	1727	0,34	0,37	
α-kessyl acetate	1743,9		0,56	0,38	0,16
8-acetoxyelemol isomer	1772,3		1,36	1,66	0,92
β-eudesmol acetate	1791,7	1792	2,93	3,01	
β-santalol acetate	1796,9	1800	1,37	1,28	2,08
kessanyl acetate	1806,7		9,65	6,13	3,67
(Z)-valerenyl acetate	1828,6			0,43	0,43
valerenic acid	1862,5	1843	3,09	1,78	0,75
hexadecanoic acid (palmitic acid)	1959,6	1951	0,34	0,42	1,59
(E)-valerenyl isovalerate	2052,1		1,54	0,88	2,32
hexadecyl isovalerate	2626,0		0,32	0,61	0,41
squalene	2795,3			0,41	0,49
Total			83,52	89,10	82,64

The cultivated valerian extracts were also characterized by the higher content of δ-elemene and lower contents of valerenic acid and borneol than the wild grown valerian extracts.

Conclusions

Higher extraction yield is obtained in SFE process from smaller particles, which indicates that the diffusion through the particle is the rate limiting process. SFE extract obtained at 10 MPa and 313 K yielded in the highest quantity of monoterpenes and sesquiterpenes. Valerenal, bornyl acetate and valerianol were dominant compounds in SFE extracts. The highest quantity of valerenal was detected in SFE extracts from Valerian III. Higher contents of valerenic acid were determined in extracts from wild grown species than in extracts from cultivated sort.

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