ISSN 1070-3632, Russian Journal of General Chemistry, 2012, Vol. 82, No. 3, pp. 595–601. © Pleiades Publishing, Ltd., 2012.
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Application of Gas-Liquid Chromatography for Standardization of Herbal Raw Materials and Herbal Drugs

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Received November 1, 2010

Abstract—Gas chromatography with mass selective detection is described as method for investigation and standardization of herbal raw materials and herbal drugs. Marker compounds and chromatographic conditions are described for some herbs.

DOI: 10.1134/S1070363212030395

Gas chromatography has come into the practice of separation, qualitative and quantitative analysis of drug raw materials, determination of active substances in manufactured drugs, and stability studies on drugs for establishing their expiry dates. Pharmaceutical analysis makes use of the gas−liquid, gas−adsorption, gas−adsorption−adsorption variants of gas chromatography.

The introduction of gas chromatography as an official method into the analytical control services of pharmaceutical enterprises is motivated by a series of advantages of this techniques, such as high sensitivity and specificity, possibility of simultaneous determination of several components of a drug (which is especially important for studies on the composition of drug herbs), and possibility of fast analysis of drug series without extra expenses.

In view of the preparation of a new addition of the domestic pharmacopoeia, Dement'eva et al. [1] developed a project of the general pharmacopoeial monograph "Gas Chromatography." In developing this regulatory document the authors introduced updates to gas-chromatographic procedures, which were developed by comparative assessment of the procedures described in the State Pharmacopoeia (XI edition) and foreign pharmacopoeias (European [2], American [3], British [4], and German [5]).

The new edition of the pharmacopoeial monograph contains assessment of the test for suitability of a chromatographic system and requirements to description of concrete analytical procedures; it allows computation of chromatographic characteristics and parameters of quantitative analysis.

Here we review research on the development of new approaches to standardization of herbal drug raw materials, with account for the requirements of the new pharmacopoeial monograph (use of modern instrumental chromatographic techniques on optimization of chromatographic conditions), as well as on the search for markers for each kind of drug herbs, both included into the Pharmacopoeia and not yet included but widely used ones. Correlations between the presence of biologically active substances in herbs and their pharmacological activity are performed.

Herbal Drug Raw Materials and Herbal Mixtures

Razhivin et al. [6] chose as objects for study three types of herbal raw materials: marsh tea (*Ledum palustre L.*), anise (*Anisum vulgare Gaerth.*), and garden sage (*Salvia officinalis L.*). These herbs are frequently included into various herbal mixtures for treating bronchitis, tracheitis, and pneumonia [7].

The composition of marsh tea is fairly well known [8–14]. Essential oils from leafy sprouts of this herb collected in different regions of the globe have quite different compositions. In particular, the principal components of the essential oils of the marsh tea from Finland, Netherlands, and European Russia are ledol, palustrol, and mircene (or mirtenal), the principal component of the essential oils of the marsh tea from Sayan Mountains and Sakhalin is *p*-cymene, and of

those from the Tom Region, limonene. It is commonly recognized that ledol is one of the principal carriers of the antitussive effect of marsh tea, and it is readily determined by gas chromatography (GC). Fursa and Korotaeva suggested a procedure for standardization of the ointment Ledum prepared from marsh tea sprouts, which is based on determination of arbutin, hydroxycinnamic acids, flavonoids, and oxidizable polyphenolic compounds.

Anise fruits contain 2.5−6% of an essential oil comprising up to 90% of anethol which partially converts into dianethol on storage. Anise essential oil exerts a mild expectorative effect and favors reflex respiratory excitation, enhanced activity of ciliated respiratory epithelium, and enhanced secretion of mucous membranes of trachea, throat, and bronchi. Anise is usually included into complex preparations (mixtures, teas). Gas chromatographic analysis of this raw material was performed in [16–20], the marker was anethol.

The principal components of garden sage essential oil are considered to be cineol (eucaliptol) and camphor [21], along with pinene, thujone, and borneol. The gas chromatographic analysis of garden sage raw materials [22–24] revealed the presence of cineol, α- and β-thujone, and borneol.

The author of the present review has studied [25] the composition of the volatile fraction of the choleretic herbal mixture no. 3 (Krasnogorsklek-sredstva OAO) and compared it with the compositions of the components of this herbal mixture. The herbal components of the mixture are as follows: wild camomile flowers (*Chamomillae flores*) (23%), mint herb (*Menthae piperitae folia*) (23%), calendula flowers (*Calendulae flores)* (23%), milfoil herb (*Achillei herba*) (23%), and tansy flowers (*Tanaceti flores*) (8%). The herbal mixture was analyzed on an Agilent 6850 Series II GC coupled with an Agilent 5973 Network mass-selective detector. GC conditions: HP-5MS column (30 m × 0.25 mm i.d., temperature program: initial 30°C, ramp rate 5°C/min, final 240°C, hold 10 min; detector temperature 200°C, injector temperature 30°C; carrier gas helium at 1 ml min^{-1}.

Achillea millefolium was studied by GC [26–32], therewith this herb was a component of an elixir [26]. Orav et al. [28] compared the compositions of the essential oils of *Achillea millefolium* collected in different European countries. The essential oil contents were 0.9–9.5 ml/kg. A total of 102 components were identified, the most abundant being sabinene, β-pinene, 1,8-cineol, linalool, α- and β- thujones, camphor, borneol, fenchyl acetate, bornyl acetate, caryophyllene, germacrene D, caryophyllene oxide, β-bisabolol, δ-cadinol, and chamasulene.

The chemical composition of calendula (*Calendula officinalis*) was studied in sufficient detail. It contains such biologically active substances as carotenoids which impart an orange color to the flowers, as well as sterines and triterpenoids [33]. Even though the composition of this drug herb is quite well known, the item "Calendula Flowers " in the State Pharmacopoeia (edition XI) contains no procedures for its qualitative and quantitative analysis. Thin-layer chromatograpgy (TLC) and spectrophotometry, recommended in [34] for flavonoids (per rutin), are insufficiently specific. The prevailing components of calendula raw material, according to the GC data in [35, 36], are sesquiterpene alcohols, specifically α-cadinol (25%).

As to tansy (*Tanacetum vulgare L.*) which is an essential-oil herb, the composition of the volatile fraction of its extracts is not yet completely understood. Sesquiterpenes and thujones were found [37–39]. Previously the most typical component of *Matricaria chamomilla* was considered to be chamazulene [7], however, with the development of gas chromatography−mass spectrometry, a series of other marker compounds was identified: α-bisabolol, *trans*-farnesol, *cis*-β-farnesene, α-cubene, and α-bisabolol oxide A [40–44].

Mentha piperita herb contains quite specific compounds: menthol, menthone, piperitone, and pulegone, which pass into herbal mixtures even at low contents of mint herb.

Dobrokhotov et al. [45] studied the composition of herbs contained in the herbal mixture applied for treatment and prevention of inflammatory diseases of parodontium. The mixture comprises calendula flowers (*Calendulae flores)*, bur beggare herb (*Herba Bidentis tripartitae*), milfoil herb (*Herba Millefolii*), oregano herb (*Herba Origani vulgaris*), burnet rootstocks and roots (*Rhizomata et radices Sanguisorbae*), licorice roots (*Radices Glycyrrhizae*), and tormentil rootstocks (*Rhizomata Tormentillae*). All the above herbs, except for burnet, are included into the State Pharmacopoeia (edition XI). Authenticity of the raw materials was recommended to be checked by microscopy (calendula flowers), quantitative reduction reactions for polysaccharides, tannins (bur beggare herb), and also

to determine the total essential oil contents (milfoil and licorice) by steam distillation.

It should be noted the above-listed authenticity tests are too general, color reactions not infrequently respond on a broad range of components of a raw materials, and commonly the total content of substances can be estimated.

The chemical composition of burnet was studied, as a rule, in terms of content of tanning substances [46].

For the markers of bur beggare, *p*-cymene, β-caryophyllene oxide, and humulene epoxide II were suggested [47–49].

Tormentil rootstocks contain tannins, primarily condensed tannins, free gallic and ellagic acids, and triterpene saponins (tormentoside, sapogenin). No information on volatile components is available.

Origanum vulgare is rich in volatile substances [50–55]. The chemical composition of another type of oregano, viz. *Origanum tytthanthum Gontsh*, was described in [56]. Seguru et al. identified certain new monoterpene derivatives in *Origanum vulgare* [57].

As applied to the herbal mixture under consideration, the following marker compounds were suggested in [45]: for hydroxy derivatives of naphthalene for calendula, furfural and 3-furaldehyde for tormentil, 5-(1,5-dimethylhex-4-enyl)-2-methylcyclohexa-1,3-diene, thujone, α-amorphene, anethol, and butan-2,3-diol for licorice root extract, bicyclic mono- and sesquiterpenoids (camphor, borneol, isoborneol, isobornyl acetate, caryophyllene, and bicyclosesquiphellandrene) for milfoil, and dimethyl sulfoxide and β-cymene (propylbenzene derivative) for bur beggare.

The component composition of the volatile fraction of the Elekasol herbal mixture (Krasnogorskleksredstva OAO) exhibiting anti-inflammatory action was studied in [58] and correlated with the compositions of the individual components of this mixture (licorice root 20%, sage leaves 20%, eucalypt leaves 20%, calendula flowers 20%, bur beggare herb 10%, and camomile 10%). Among the marker compounds, diversity and information content are characteristic of terpenes and terpenoids, naphthalene derivatives (characteristic, in particular, of calendula raw material), and azulene and furan derivatives. Standardization of the mixture by the typical components of licorice root is complicated by the fact that the results of their determination are not enough reproducible, most likely because of the low content in the raw material. At the same time [59], in the analysis of the multicomponent mixture, such specific markers of licorice root as 5-(1,5-dimethylhex-4-enyl)-2-methylcyclohexa-1,3-diene, thujone, α-amorphene, anethol, and butan-2,3-diol were identified, which extends the possibilities of standardization of the mixture. Of particular interest, in terms of standardization, is anethol, the principal component of the essential oil of a number of *Apiaceae* family plants: By optimizing chromatographic conditions, one can determine this compound with a high precision and thus assess the quality of the mixture.

Razhivin et al. [60] studied the composition of the liquid extracts of the herb drug raw material used to prepare complex medicines for kidney and urinoexcretory system. The aim of this work was to study certain herbal mixtures and phyto teas from different producers for their quality assessment. The GC−MS analysis of forty pharmacopoeial drug herbs showed that 14 herbs contain quite specific compounds inherent exclusively in a concrete raw material [61].

Essential Oils

Gas chromatography provides the most reliable assessment of the authenticity of essential oils [62–65].

As a rule, in studying the component composition of essential oils, there is no necessity to isolate individual components: This is only necessary for first identified compounds [9]. However, successful identification is possible, when databases including principal analytical parameters of the analytes are available. In the absence of such a database, the experimenter has to use a series of reference compounds. This approach is still widespread, but it does not prevent from mistakes in identification, associated with accidental coincidence of the analytical characteristics of individual compounds amd components of complex mixtures.

The lack of informational tools for the chromatographic analysis of essential oils is, to certain extent, compensated for the work of Zenkevich [66]. The author systematized a minimum set of GC and MS data for mono- and sesquiterpene hydrocarbons, necessary and sufficient for solving the practical tasks of identification of these components in herbal essential oils.

Standardization of production, pharmaceutical inclusive, is accomplished by the results of GC

analysis using stationary phases which ensure interlaboratory reproducibility of retention index. The State Standard 14618.0-78 recommends nonpolar poly (dimethylsiloxane) (PMDS) elastomers and polar poly (ethylene glycols) with MW 400–20000 [67]. Chromatographic columns can be both packed and capillary, the latter preferred, oven temperature 50–300°C (programmed temperature modes are recommended).

Concrete chromatographic conditions should be given in a special pharmacopoeial description which should include the procedure of quantitative determination of one or several prevalent or specific components of the essential oil (marker compounds) and specify their reference contents. In cases where reference sample of individual components are available, GLC can be used for authenticity testing and qualitative analysis [57].

Pisarev and Denisenko [68] made use of GLC to study the component composition of essential oils of *Juniperus oblonga*. Analysis was performed on an HRGC Mega Series instrument with FID-40 and an SPB-5 quartz column (15 m×0.53 mm). The oven temperature was programmed from 60 to 250°C at a rate of 2°C/min, injector temperature 230°C, detector temperature 250°C, carrier gas helium, sample volume 1 μm. The logarithmic retention indices were calculated using C_9–C_{20} *n*-alkanes as a reference series. It was shown that the essential oil of needles of *Juniperus oblonga* contains 17 compounds, and that of fruits contains 18 compounds, of which 4 are present at a trace level. The principal component of the essential oil of needles is α-pinene (41.4%), and the principal components of the essential oil of fruits are α-pinene and sabinene (30 and 25.4%, respectively).

The essential oil of another juniper species was studied in [69].

The essential oil of *Artemisia jacutica Drob.* (stationary phase PMDS) was found to contain chamazulene (20–30%), β-farnesene, α-gumulene, δ-cadinene, and bisabolol [70]. Analysis of the essential oil of silky wormwood [71] revealed limonene, β-phellandrene, cineol, g-terpinene, thujone, and β-caryophyllene. The GC–MS analysis of *Artemisia rehan* in [72] revealed 22 components, the principal of which were camphor and davanone, and eudalene was also found. The presence of davanone was also mentioned in [73]. Tropnikova et al. [74] studied the composition and antimicrobial activity of five *Nepeta* species (*N. bucharica, N. grandifora, N. nuda, N. sibirica*, and *N. transcaucasica*) cultivated in the Leningrad Region.

Reliability of identification of essential oil components was suggested to assess using hexane-acetonitrile partition coefficients [75].

Elixirs, Extracts, Tinctures, and Homeopathic Preparations

Elixirs and balsams contain complex mixtures of biologically active compounds, some of which are present at nearly trace levels (10^{-4}–10^{-6} g l^{-1}). An important role in such formulations belongs both to volatile and to semi- or nonvolatile components of herb raw materials. Gas chromatography (analysis of volatiles) and liquid chromatography (analysis of nonvolatiles) can be efficient for standardization of elixirs and balsams only if samples are prepared in an optimal way. In developing sample preparation procedures for each of the above instrumental methods one has to take account of specific features of each preparation. Various procedures of sample preparation are described in [76].

Makarov et al. [77] studied the composition of volatile components of the elixir Demidovskii by means of GC–MS and GC–FID. This elixir has diverse and multicomponent compositions both at the level of raw material and at the level of individual compounds whose concentrations in the elixir are fairly low (10^{-4}–10^{-6} g l^{-1}).

The volatile components of the elixir were isolated by steam distillation, after which they were extracted from the aqueous-alcoholic distillate with diethyl ether. The same operation was performed with all aqueous-alcoholic solutions obtained separately for all raw materials, as well as for their mixture.

Reshetnyak et al. [78] demonstrated the possibility of GLC standardization of the eleuterococcus extract. This extract contains a great number of oxygen-containing compounds (phenolic compounds, furan derivatives, and hydroxycinnamic acids) which are efficiently analyzed by this method. Vinylguaiacol can be used as the marker compound.

Gas–liquid chromatography has also found application in standardization of homeopathic matrix tinctures [79–81]. Kostennikova and Kopyt'ko [79] made use of a Varian ion-trap GC–MS system with a DBWAX quartz capillary column (30 m × 0.25 mm, film thickness 0.25 μm) in the following conditions:

carrier gas helium at 1.5 cm^3 min^{-1}; injector temperature 220°C; oven temperature program: initial 40°C (hold 2 min), ramp 15°C/min to 120°C, and ramp 5°C/min to 230°C (hold 10 min); split ratio 1:20; sample volume 0.3 μl. Each sample was analyzed no less than 3 times.

Kopyt'ko et al. [80] studied the matrix tinctures from common club-moss spores, obtained by a homeopathic technology [81]. Volatile compounds and fatty acids of the lipid fraction from the matrix tincture of lycopodium were analyzed on a Saturn 3 ion-trap GC−MS system with a DBWAX quartz capillary column (30 m × 0.25 mm, film thickness 0.25 μm) in the following conditions: carrier gas helium at a rate of 1.5 cm^3 min^{-1}; injector temperature 240°C; oven temperature program: initial 35°C (hold 3 min), ramp 20°C/min to 90°C, ramp 3°C/min to 230°C (hold 27 min); split ratio 1:20; sample volume 0.5 μl.

Sample preparation was as follows: 10 ml of the matrix tincture of lycopodium was extracted with 10 × 2 ml of hexane (each portion was shaken for 10 min); the extracts were combined and evaporated to dryness on a rotary evaporator in a vacuum at ~50°C, and the residue was dissolved in 2 ml of ethyl acetate.

According to the resulting data, the tincture contains no less than 18 fatty acids. Among saturated acids prevailing are palmitic and behenic acids; the unsaturated acids found are linolic and linolenic.

In general, GLC is quite a perspective technique for standardization of herbal drug raw material, herbal mixtures, as well as dosage forms prepared from herbal raw material. The high sensitivity and selectivity allow application of this method for qualitative and quantitative phytochemical analysis.

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