A.A. Filippova et al /J. Pharm. Sci. & Res. Vol. 11(1), 2019, 144-146

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Development of Geraniol Quantitation Method in Oral Rinse "Splat Medical Herbs" Containing *Pelargonium Graveolens* Essential Oil by GC-FID

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Abstract:

The article presents a novel method of geraniol quantitative analysis as a characteristic component in the geranium (*Pelargonium graveolens* L'Hér) essential oil of the oral rinse formulation. The proper quality of the product was confirmed by the established concentration of natural geraniol (0.007%), declared by the manufacturer in accordance with the requirements of TR TS-009-2011. A method has been developed for the quantitative determination of geraniol in the oral rinse containing geranium essential oil according to a new proposed indicator "mass fraction of a characteristic component". Inclusion of this new indicator in the developed interstate and state standards, listed in TR TS-009-2011, was recommended; as well as the inclusion of the natural geraniol of geranium essential oil in the substances list of Appendix No 2 (TR TS-009-2011) was suggested.

Keywords: oral rinse, essential oil, *Pelargonium graveolens*, geraniol, gas chromatography, flame ionization detection, characteristic components, oral hygiene products, medicinal plant raw materials.

INTRODUCTION

At present, the quality of oral hygiene products (OHPs) is determined by the technical regulations of the Customs Union (TR TS-009-2011) within the framework of the Eurasian Economic Union (EAEU), which is in force in all member countries (EAEU member states). This regulatory document contains mandatory requirements for the product safety, the compliance with them is a prerequisite. Evaluation (confirmation) of the above-mentioned OHP compliance with these requirements allows these products to be released to the market [1].

Among the main nine requirements, special attention is paid to the chemical composition and physico-chemical parameters. This interest is partly aroused by the expansion of the OHPs range due to the inclusion of new components in the composition, including those of plant origin. However, new OHPs entry into the perfumery and cosmetic market should be accompanied by the development of new requirements governing the quality of these products, i.e. standardization [2].

Tooth pastes and oral rinses, which include treatment-and-prophylactic additives, such as various extracts, essential oils (EOs), and individual biologically active substances (BAS), hold a special place in the preventive OHPs market. This is due to the increased interest of the modern consumer in effective, but safer phytoprophylaxis, used to maintain individual oral hygiene [2-7].

Of all the known herbal additives in the formulation of OHPs, EOs are emited. EOs are comparable in their antibacterial effectiveness to such synthetic analogues as chlorhexidine and triclosan, but EOs are safer [8-11]. The EO content in the OHPs formulation is not regulated due to the complexity of EOs composition. However, EOs activity is due to the presence of individual BAS in a certain amount. Similar information is given in TR TS-009-2011, but only for some similar synthetic BAS [1], this information is not available for natural BAS. So for synthetic geraniol, the main characteristic substance of geranium essential oil (GEO), the range of normalized values is defined, within which it exerts its prophylactic antibacterial action [1]. However, no one carried out an product quality assessment of the OHPs containing GEO, especially quantitative determination of natural geraniol. For this reason, the objective of this experiment was the method development for quantitative analysis of geraniol in the oral rinse "SPLAT Medical Herbs" according to the new proposed indicator "mass fraction of the characteristic component". The aim of this study was to develop a methodology for the quantitative determination of geraniol in the oral rinse containing GEO, and further recommendations for the inclusion of this new indicator in the developed interstate and state standards listed in TR TS-009-2011.

METHODS

The oral rinse Professional series "SPLAT Medical Herbs" (GOST R 51577-2000, TR TS 009/2011) was used as the object for study. 5 samples of one series ("011119" means the batch No. / month / year, respectively) have been analyzed.

Oral rinse "Medical Herbs" is a solution of emerald color for local administration. According to the recipe, it contains such excipients as purified water, glycerin, glutamate diacetate tetrasodium, alcohol, benzyl alcohol, polyglyceryl-4 laurate / sebacate, polyglyceryl-6 caprilate / caprate, coco-sodium sulfate, sodium benzoate, flavoring, potassium sorbate, carboxymethylcellulose, citric acid, papain, maltitol, calcium lactate, sodium hydroxide, potassium thiocyanate, lactoferrin, lactoperoxidase, glucooxidase, glucose pentaacetate, CI 75810 (chlorophyllin copper complex - natural green coloring substance), without fluoride and following treatment-and-prophylactic additives:

• aqueous alcoholic extracts of chamomile flowers (*Chamomillae recutitae flores*) and sage leaves (Sal*viae officinalis folia*), stevia extract (*Steviae rebaudianae folia*), licorice roots extract (*Glycyrrhizae glabrae radices*), hawthorn flowers extract (*Crataegi monogynae flores*), Sea buckthorn fruit extract (*Hippophaes rhamnoides fructus*).

• Geranium herb essential oil (*Pelargonii graveolentis herba*), camphor tree bark essential oil (*Cinnamomi camphorae cortex*).

Equipment and reagents

The "Crystallux-4000M" gas chromatograph (Meta-Chrom, Russia) was used in the analysis; the following conditions were applied: chromatographic column – HP-5ms capillary quartz column (Agilent Technologies, USA), 30 m × 0.25 mm, 0.25 µm with bonded phase (5% phenyl-95% -methylpolysiloxane); mobile phase – nitrogen; carrier gas flow rate – 30 cm^3/min - 30 cm^3/min - 60 cm^3/min; vaporizer temperature – 200 °C; column temperature gradient – from 100 ˚C to 150 ˚C, heating 5 ˚C / min; pressure on capillary column – 1 atm; detector – FID (flame ionization detector), detector temperature – 250 °C; hydrogen

flow rate – 35 cm^3/min; air flow rate – 350 cm^3/min; injection sample volume –1 µl.

Ethyl alcohol 95% (analytical grade) was used for solutions preparation, geraniol standard sample (Sigma-Aldrich Cat. No. 48798) was used as analytical standard.

Results And Its Discussion

The preparation of *geraniol standard sample solution for testing of the chromatographic system suitability*: about 0.1757 g (precisely weighed amount) of geraniol standard sample (Sigma-Aldrich cat. No. 48798) was placed in a 25 ml volumetric flask, dissolved in 5 ml of 95% ethyl alcohol, solution volume was adjusted to the mark with 95% ethyl alcohol and mixed. The solution was used freshly prepared.

The preparation of the *rinse test solution*: 5 ml of 95% ethyl alcohol was placed in a 25 ml volumetric flask with a glass stopper, 5 ml of oral rinse "SPLAT Medical Herbs" was added. The resulting solution was filtered through a paper filter into a 25 ml volumetric flask and the volume of the filtrate was adjusted to the mark with same solvent.

1 µl of the standard sample solution and the test solution were sequentially chromatographed. A fivefold test of each solution was performed to obtain statistically reliable data.

Checking the chromatographic system suitability.

The chromatographic system is considered suitable if the following conditions are met:

✓ chromatographic column efficiency, calculated from the peak of geraniol, it is not less than 50261.09 theoretical plates;

✓ peak asymmetry factor – 0.662;

✓ relative standard deviation of peak areas is not more than 2.64%.

Retention time: geraniol – about 6.00 min. In accordance with Figure 1.

Identification of geraniol in the rinse test solution was performed by the retention time of geraniol in the geraniol standard sample solution.

A general view of the GC-FID-chromatogram of the rinse test solution with detected chromatographic peaks is shown at Figure 2.

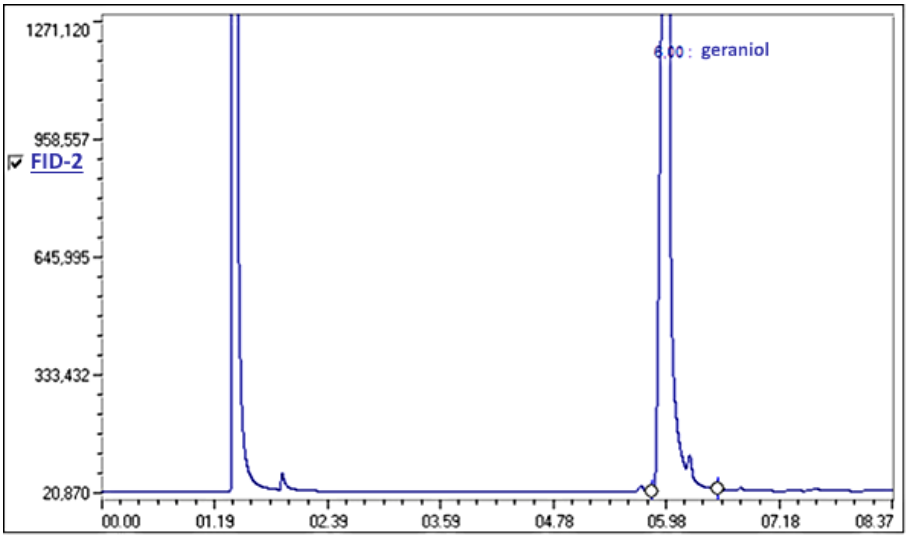

Figure 1 – GC-FID-chromatogram of geraniol standard sample solution.

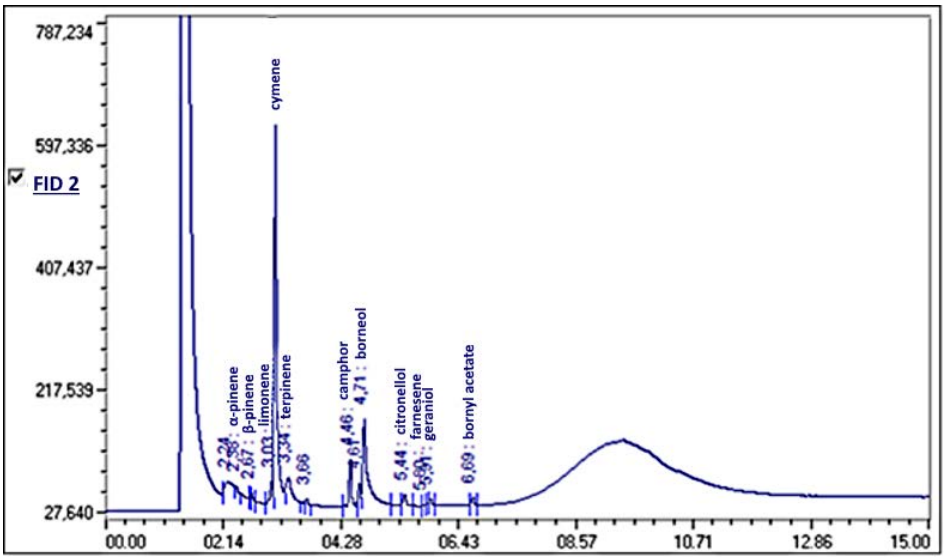

Figure 2 – GC-FID-chromatogram of oral rinse "SPLAT Medical Herbs" test solution.

Table 1 - The results of determining the geraniol concentration in oral rinse “SPLAT Medical Herbs”.

Rinse sample No.	Geraniol peak area, mV * min	Geraniol peak height, mV	Retention time, min	Geraniol concentration, mg/100 g
1.	0.2887	7.457	5.91	7.11
2.	0.2877	7.456	5.91	7.08
3.	0.2867	7.455	5.90	7.06
4.	0.2859	7.454	5.90	7.04
5.	0.2900	7.458	5.92	7.13

Statistical data processing (geraniol concentrations)							
X (∑X/5)	S^2	S	±Δ	X+Δ	X-Δ	E	E%
7.084	0.00133	0.0365	0.0453	7.129	7.039	0.0064	0.64

Geraniol is a characteristic substance, predominant or priority substance marker of analyzed sample containing GEO. GC-FID-analysis was performed by the method of external standard. The content of geraniol was calculated by the following formula:

$$X = \frac{S_{test}}{S_{st}} \cdot \frac{m_{st}}{V_{st}} \cdot \frac{V_{test}}{m_{test}} \cdot 100\% \cdot 1000, \text{ where}$$

S_{test} – geraniol peak area at the chromatogram of the rinse test solution (mV * min);

S_{st} – geraniol peak area at the chromatogram of a geraniol standard solution (mV * min);

m_{test} –sample weight of the test sample (g);

m_{st} – standard sample weight (g);

V_{test} –dilution volume of the test sample (ml);

V_{st} – the dilution volume of the standard sample (ml).

The geraniol content in the rinse test solution during the GC-FID analysis was 7.08 ± 0.0453 mg / 100g (0.007%). All calculations are shown in Table 1.

CONCLUSION

The concentration of natural geraniol in the oral rinse “SPLAT Medical Herbs” containing GEO has been established; it averaged 0.007%. This geraniol content is sufficient to provide a preventive antibacterial effect for oral biofilms. The resulting concentration of geraniol in the studied OHP corresponds to the range of normalized values [0.001%; 001%] of synthetic geraniol with number 78 given in substances list of Appendix No 2 in TR TS-009-2011. Quantitative determination of geraniol as a characteristic substance of GEO in the OHP composition confirmed the proper quality of the product declared by the manufacturer in accordance with the requirements of TR TS-009-2011.

A GC-FID method has been developed for the quantitative determination of geraniol in the oral rinse containing GEO according to a new proposed indicator “mass fraction of a characteristic component”. Inclusion of this new indicator in the developed interstate and state standards, listed in TR TS-009-2011, was recommended; as well as the inclusion of the natural geraniol of GEO in the substances list of Appendix No 2 (TR TS-009-2011) was suggested.

ACKNOWLEDGEMENT

This research was supported by the “Russian Academic Excellence Project 5-100”.

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