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GAS CHROMATOGRAPHIC ESTIMATION OF AROMATIC HERBAL DRUG CONTENT IN THEIR MIXTURES

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Summary

Gas chromatographic method was developed for estimation of aromatic herbal drug content in their mixtures subjected to application in bakery industry. Method includes isolation of essential oil from single herbal drugs and their mixtures, GC analysis of isolated oils, selection of reference constituent in GC profile of single oils, and calculation of the contents of constitutive herbal drugs in their mixtures. With certain precautions this approach could be successfully applied in current quality control practice of such and similar products.

Keywords: *herbal drug, mixtures, determination, essential oils, gas chromatography*

Introduction

When certain product consists of the mixture of several cut or pulverised herbal drugs, or appropriate plant extracts, the most often is very difficult to accomplish required quantitative analysis. Quantification in this case assumes estimation (or determination) of contents of constitutive single herbal drugs (or appropriate extracts). Although modest direction for the quantification, which can be found in the most common reliable sources, offering and pointing at procedures that includes work with certain herbal drug fingerprints, it is not quite clear how to achieve this goal.

From the other side, mentioned fingerprints of medicinal and aromatic plants, the most often obtained by certain chromatographic techniques, such as, thin-layer chromatography (TLC), gas-liquid chromatography (GC), or high performance liquid chromatography (HPLC), are variable. This variability is closely related with the chemical composition of analysed herbal drugs (or extracts), which can vary in very wide proportions. Subsequently, it is quite clear that virtual analytical standards in targeted quantification could be only constitutive original single herbal drugs (or extracts), used for preparation of finished product.

Furthermore, in each of fingerprints (chromatographic profiles) of single herbal drugs (or extracts), appropriate marker constituent should be selected (as a reference) for quantification purposes. All these markers must present specific constituents for selected drugs (extracts) for targeted mixture, and should be easily identified, and quantified in it. Then, after conducting sharply defined procedures for obtaining certain fingerprints (chromatographic profiles) of all standards and samples, and properly applying basic quantification rules for processing of raw chromatographic data, successive estimation of constitutive herbal drugs (or extracts) in their mixtures could be expected.

In this article, results of determination essential oils in homogeneous mixtures of pulverised herbal drugs and starting (original) pulverised single herbal drugs were used, along with those coming out form accompanied GC analyses, for single drug estimation in their mixtures.

Proposed procedure consisted of the following steps: isolation of essential oil from single herbal drugs and their mixtures (1°), GC analysis of isolated oils (2°), selection of reference (marker) constituent in GC profile of each single oil (3°), and calculation of the contents of constitutive herbal drugs in their mixtures (4°).

Material and methods

Samples selection

Subjects of the characterisation were two herbal mixtures, which contained, along with one non-aromatic drug, two or three pulverised aromatic herbal drugs, as constitutive (single) herbal drugs. The first of these contained 35% of *Frangulae cortex pulvis*, 20% of pulverised mint leaf (*Menthae piperitae foilum*), 20% of caraway fruit (*Carvi fructus*) and 25% of parsley fruit (*Petroselini fructus*). The second one contained of 15% of *Cynarae folium pulvis*, 55% of pulverised oregano leaf (*Origani heracleotici folium*) and 30% of coriander fruit (*Coriandri fructus*).

Isolation of essential oils

Essential oils were isolated and quantified in three repetitions in a Clevenger type apparatus, according to Ph. Jug. IV.

Analytical gas chromatography (GC/FID)

GC/FID analysis of the oils was carried out on a Hewlett-Packard HP-5890 Series II GC apparatus, equipped with split-splitless inlet and automatic liquid sampler (ALS), attached to HP-5 column (25 m · 0.32 mm, 0.52 μm film thickness) and fitted to flame ionisation detector (FID). Carrier gas flow rate (H_2) was 1 ml/min, split ratio 1:30, injector temperature was 250°C, detector temperature 300°C, while column temperature was linearly programmed from 40-260°C (at rate of 4°/min). Solutions of essential oil samples in ethanol (¨1%) were consecutively injected by ALS (1 μl, split mode) in triplicate. Area percent reports, obtained as result of standard processing of chromatograms, were used as base for the quantification purposes.

Gas chromatography - mass spectrometry (GC/MS)

The same analytical conditions (as those mentioned for GC/FID) were employed for GC/MS analysis, along with column HP-5MS (30 m · 0.25 mm, 0.25 μm film thickness), using Hewlett-Packard HP G 1800C Series II GCD system. Instead of hydrogen, helium was used as carrier gas. Transfer line was heated at 260°C. Mass spectra were acquired in EI mode (70 eV), in m/z range 40-450. Sample solutions in ethanol (¨1 %) were injected by ALS (200 nl, split mode).

Selection of marker constituents

While selecting marker constituents in each of single oils, the aim was to select the most abundant and the most specific components, whenever it was possible. Although the final calculation could be theoretically accomplished taking from GC profile of selected oil component of free choice, for this purpose the major oil constituents were typically selected. Marker constituents for selected herbal drugs and related oils, in the case of this study, were menthol for mint, carvone for caraway, myristicin for parsley, carvacrol for oregano and linalool for coriander.

Results and discussion

Results on the essential oil content, as well as the contents of selected marker constituents are presented in Table 1. Simultaneously, normalised chromatograms (GC) of essential oils isolated from test mixtures and their constitutive herbal drugs are given in Figures 1 and 2.

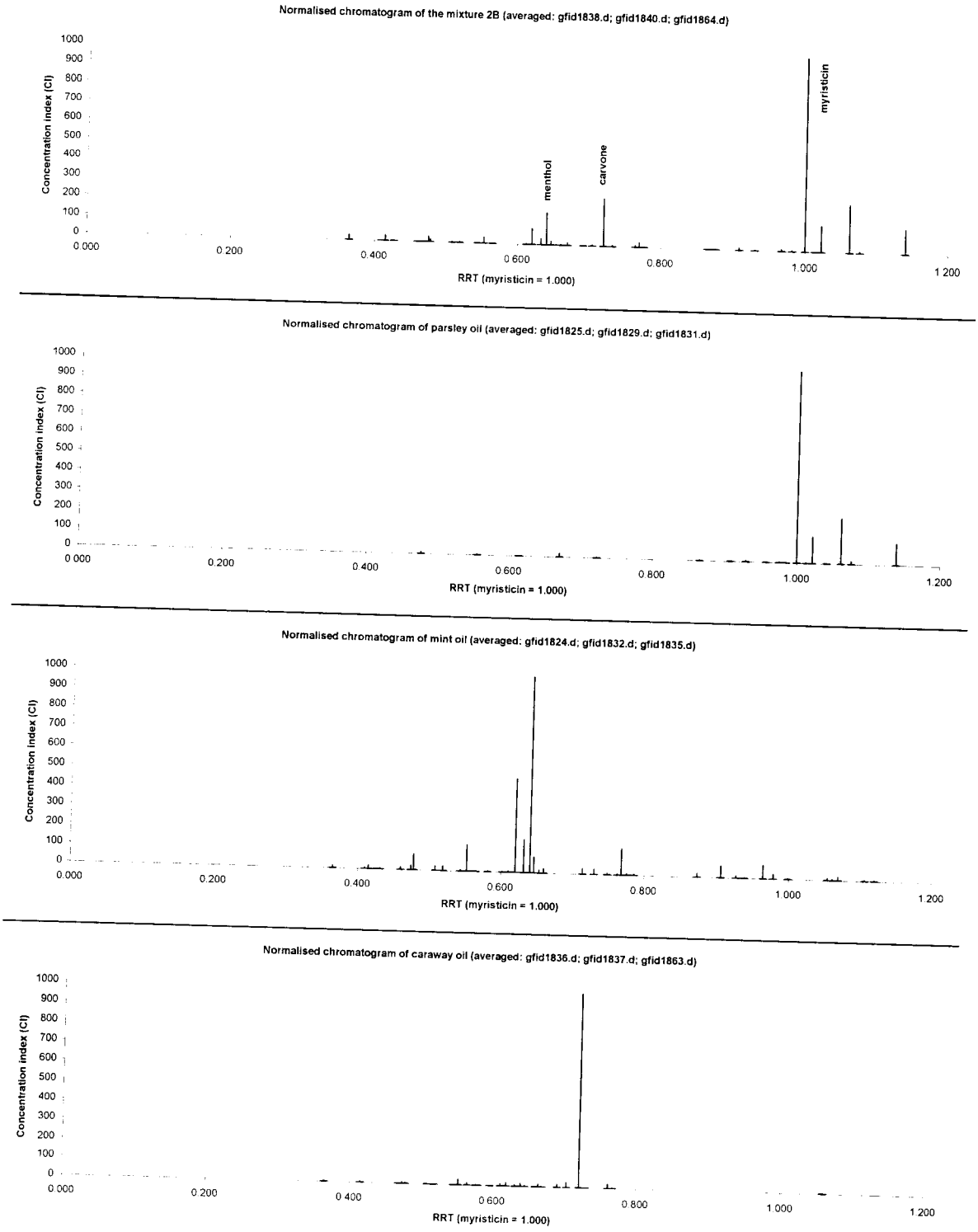

Fig. 1. Normalised chromatograms of the essential oils of mixture 2B and its constitutive aromatic herbal drugs (mint leaves, caraway seeds and parsley seeds)

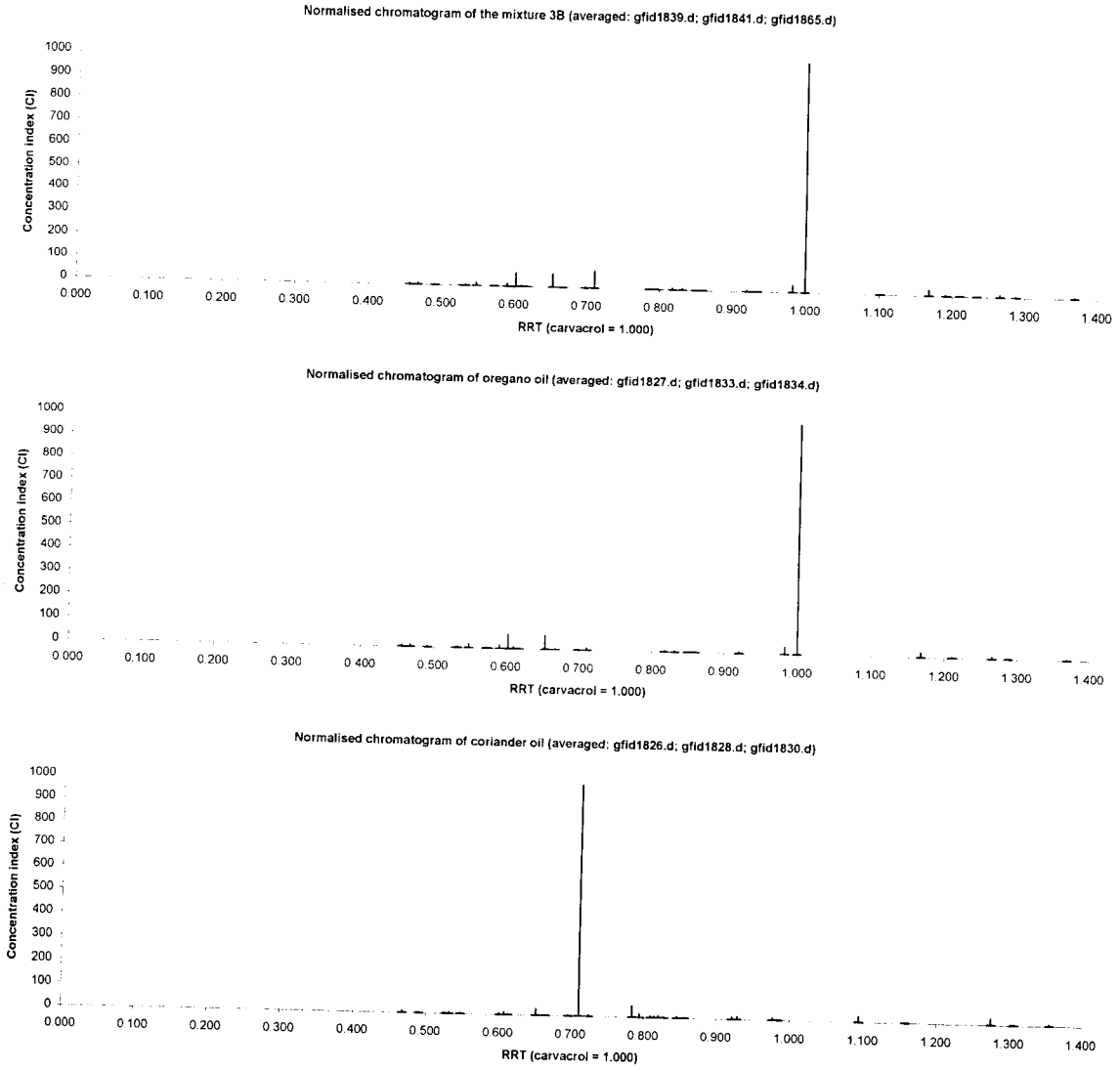

Fig. 2. Normalised chromatograms of the essential oils of mixture 3B and its constitutive aromatic herbal drugs (oregano leaves and coriander seeds)

Table 1. Average contents of essential oils and related marker constituents in test samples

Sample	Content of the oil (%)	Marker content (%)	Remark
Coriander	0.31	79.97	linalool
Parsley	0.64	64.60	myristicin
Mint	0.78	39.04	menthol
Oregano	3.20	78.83	carvacrol
Caraway	0.39	82.70	carvone
Mixture 2B	0.88	7.19 10.50 43.29	menthol carvone myristicin
Mixture 3B	1.52	5.53 73.49	linalool carvacrol

Tentative calculation procedure assumed following approximations: content of the essential oil in non-aromatic drugs coming into composition of herbal mixtures (*Frangulae cortex* and *Cynarae folium*) is zero (AP1), and relative density of all essential oils isolated from different single herbal drugs and mixtures is identical and equal to 1 (AP2).
Content of essential oils of single herbal drugs ($\%EO_x$) in the essential oils of herbal mixtures was calculated according to the equation [Eq.1], where M_m and M_x are concentrations of selected marker constituent (expressed in percents) in the oil from mixture and corresponding pure oil, respectively.

$$\%EO_x=100\cdot M_m/M_x \qquad \text{[Eq.1]}$$

Content (% m/m) of single herbal drugs (%DX) in appropriate mixtures could be calculated from the equations [Eq.2], or [Eq.2a], where $\%EO_m$ is the content of oil in herbal mixture and $\%ED_x$ - content of the oil in the single herbal drug (X).

$$\%DX=100\cdot(\%EO_m/\%ED_x)\cdot(M_m/M_x) \qquad \text{[Eq.2], e.g.}$$

$$\%DX=100\cdot(\%EO_m\cdot M_m)/(\%ED_x\cdot M_x) \qquad \text{[Eq.2a]}$$

Simultaneously, sum of contents of all constitutive herbal drugs in their mixture ($\%DX_1$, $\%DX_2$, ..., $\%DX_n$), should be 100, as is presented in equation [Eq.3].

$$\%DX_1+\%DX_2+ \ldots+\%DX_n=100 \qquad \text{[Eq.3]}$$

Table 2. Content of the oils of mint, caraway and parsley in mixture 2b essential oil

Constituents	Content (% m/m)	
	Expected*	Found**
Menthae piperitae aetheroleum	39.59	18.42
Carvi fructi aetheroleum	19.80	12.70
Petroselini fructi aetheroleum	40.61	67.01
Total:	100.00	98.13

*According to determined content of essential oil in single herbal drugs.
** According to calculation using equation [Eq.1] (uncorrected).

Table 3. Content of the oils of oregano and coriander in mixture 3b essential oil

Constituents	Content (% m/m)	
	Expected*	Found**
Origani aetheroleum	95.08	93.23
Coriandri fructi aetheroleum	4.92	6.91
Total:	100.00	100.14

*According to determined content of essential oil in single herbal drugs.
** According to calculation using equation [Eq.1] (uncorrected).

Table 4. Content of mint, caraway and parsley in mixture 2b

Constituents	Content (% m/m)		
	Declared	Found*	Found**
Frangulae cortex (pulvis)	35.00	-	36.49
Menthae pip. folium (pulvis)	20.00	20.77	9.30
Carvi fructus (pulvis)	20.00	28.64	12.83
Petroselini fructus (pulvis)	25.00	92.43	41.38
Total:	100.00	141.84	100.00

*EO_m is taken from Table 1 and %DX is calculated from [Eq.2], without correction in [Eq.3].
**EO_m is calculated as corrected (proportional) sum of constitutive oils and %DX is calculated from [Eq.2].

Table 5. Content of oregano and coriander in mixture 3b

Constituents	Content (% m/m)		
	Declared	Found*	Found**
Origani folium (pulvis)	55.00	44.28	53.93
Coriandri fructus (pulvis)	30.00	33.88	41.29
Cynarae folium (pulvis)	15.00	21.84	4.78
Total:	100.00	100.00	100.00

*EO_m is taken from Table 1 and %DX is calculated from equation [Eq.2].
**EO_m is calculated as corrected (proportional) sum of constitutive oils and %DX is calculated from [Eq.2].

Discussion about above presented results should take into account the aim of developed procedure, for quality control of herbal mixtures suggested for use in bakery industry in relatively low concentrations (up to 2-3%).

The first approximation in our calculation (AP1) assumes that non-aromatic drugs (*Frangulae cortex* and *Cynarae folium*) which come into composition of herbal mixtures do not contain essential oils at all. In opposite, certain deviation of obtained results to those expected, could be occurred. According to the second approximation (AP2), relative densities of all essential oils coming into account are identical and equal to 1, what is surely not true. Typical values for relative densities of oils taken into account ranging from a 0.900-0.916 for mint, 901-920 for caraway, 1.043-1.083 for parsley, 0.935-0.960 for oregano, and 0.862-0.878 for coriander, what means that density could vary (roughly) from 0.86-1.08, or about 20%. Subsequently, it is quite clear that AP2 itself could be the source of rather significant deviations.

Furthermore, procedure for isolation and determination of the essential oil of parsley (gravimetric), differed from that applied in the case of all other samples (volumetric). Because of nature of procedure applied in the case of parsley, there is a certain suspicion that composition of oil is somewhat changed, due to possible decrease of contents of low volatile constituents, during "drying" of oil.

Cause of deviations could be also found in non-uniformity of samples tested (first of all herbal mixtures), imprecision in standard procedure applied for isolation and quantification of essential oils, errors coming out from GC analyses, as well as poor selection of marker constituents.

Conclusions

Results obtained approved our assumption that this approach could be successfully applied in current quality control practice of such and similar products. However, certain precautions should be applied especially in the part dealing with the essential oil quantification and isolation.

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