CHROMSYMP. 140

DIRECT HEADSPACE GAS CHROMATOGRAPHIC ANALYSIS WITH GLASS CAPILLARY COLUMNS IN QUALITY CONTROL OF AROMATIC HERBS

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SUMMARY

A simple procedure, previously set up for the quick evaluation of aromatising herbs by direct headspace gas chromatographic analysis, was checked with regard to its application as a routine method. Many samples of herbs and drugs of various kinds and of different qualities were subjected to a sensory evaluation and to headspace analysis. There are significant analytical differences between herbs of good and inferior quality in agreement with the organoleptic evaluation of the same drugs, judged by an experienced panel of tasters. Some tests were performed to determine the exact content of each substance present in the headspace of the examined herbs: preliminary results are given. A suitable internal standard was used for quantitative purposes. The proposed technique is in accordance with the requirements of the Quality Control Service.

INTRODUCTION

Headspace gas chromatography (HS-GC) is a powerful tool for solving particular and difficult problems, such as trace analysis, or for checking a wide variety of diverse materials[1]. The advantages of this technique allowed us to set up a very useful method for the requirements of our Marketing and Production Services.

In previous work[2], we have described a simple procedure for the quick evaluation of aromatizing herbs and drugs by direct HS-GC analysis with capillary columns. This method is very suitable for the qualitative control of these spices, owing to its rapidity and its non-destructive nature; the sample composition or the compounds contained in the herb are unchanged, unlike in other techniques, such as distillation and extraction by organic solvents. Furthermore, we noticed that the aromagrams obtained by means of HS analyses resembled more closely that of the flavour of the herb than those derived from essential oils analyses[3].

Following these interesting results, we undertook a screening of the herbs which are of interest to us; many samples of aromatic drugs of various kinds and different qualities were therefore checked in order to evaluate the general effectiveness of the method, and to verify its practical application as a routine methodology.

Since at present sensory evaluation is the most effective although subjective, method we also compared the analytical results with the organoleptic ones. Indeed a sounder judgement about the quality of the examined herb could be achieved by con-

firming the organoleptic criteria with data founded on more reproducible and objective values.

EXPERIMENTAL

At first, each herb was examined by a panel of tasters, who gave an opinion about its quality. Usually, this evaluation includes a visual appruised of the sample (storage conditions, incidental deteriorations, etc.) and a sensory appraisal of its olfactory properties. In this connection it is very important to point out that we must distinguish between the intensity and the quality of the odour. A Sample with a strong flavour may be rejected owing to the coarseness of its olfactory properties. Anyway, this type of herb is not suitable for our purposes, because it is not well balanced with the organoleptic properties of the other ingredients in a product.

The same samples were then subjected to HS-GC analysis in order to obtain the related aromagrams. A suitable sample of the herb was finely milled in a glass grinder for a fixed time, avoiding overheating; a weighed amount of the ground herb (usually 1 g) was then put in a glass vial, sealed with a PTFE–rubber septum and finally crimped by an aluminium cap. After conditioning in a thermostat bath for 1 h at 60°C, the HS-GC analysis was performed on a Carlo Erba 2920 gas chromatograph, coupled with an automatic sampler Model HS 250.

We used the following operating conditions: column, 50 m, OV-1, 0.15-μm stationary phase thickness; carrier gas; hydrogen, flow-rate 1.9 ml/min; splitting ratio, 1:4; injector and flame ionization detector temperatures, 200°C; oven temperature, from 20°C to 150°C at a rate of 3.5°C/min; quantity injected 1 ml; attenuation, $\times$ 32; sampler syringe temperature, 80°C; sampler bath temperature, 60°C.

The data were processed by a Perkin-Elmer integrator, Model Sigma 10.

Table I shows the names of the herbs examined and their related sensory evaluations, and Fig. 1 shows the corresponding chromatograms obtained. Chromatograms a are from the least valuable sample, chromatograms b from the most valuable ones.

TABLE I

HERBS EXAMINED

+ + + = Very good quality; + + = good quality; + = poor quality; – = very poor quality; — = bad quality.

No.	Botanical name	Common name	Scores from sensory evaluation	
			a	b
1	Achillea millefolium	Yarrow	–	+ +
2	Angelica Arcangelica	Garden Angelica	–	+ + +
3	Artemisia Absinthium	Common Wormwood	+	+ + +
4	Artemisia pontica	Roman Wormwood	–	+ +
5	Citrus aurantium v.a.	Bitter Orange tree	+	+ + +
6	Citrus limonum	Lemon tree	—	+ +
7	Coriandrum sativum	Coriander	—	+ + +
8	Hypericum perforatum	St. John's wort	+	+ + +
9	Mentha piperita	Peppermint	+	+ + +
10	Satureja hortensis	Savory	–	+ +
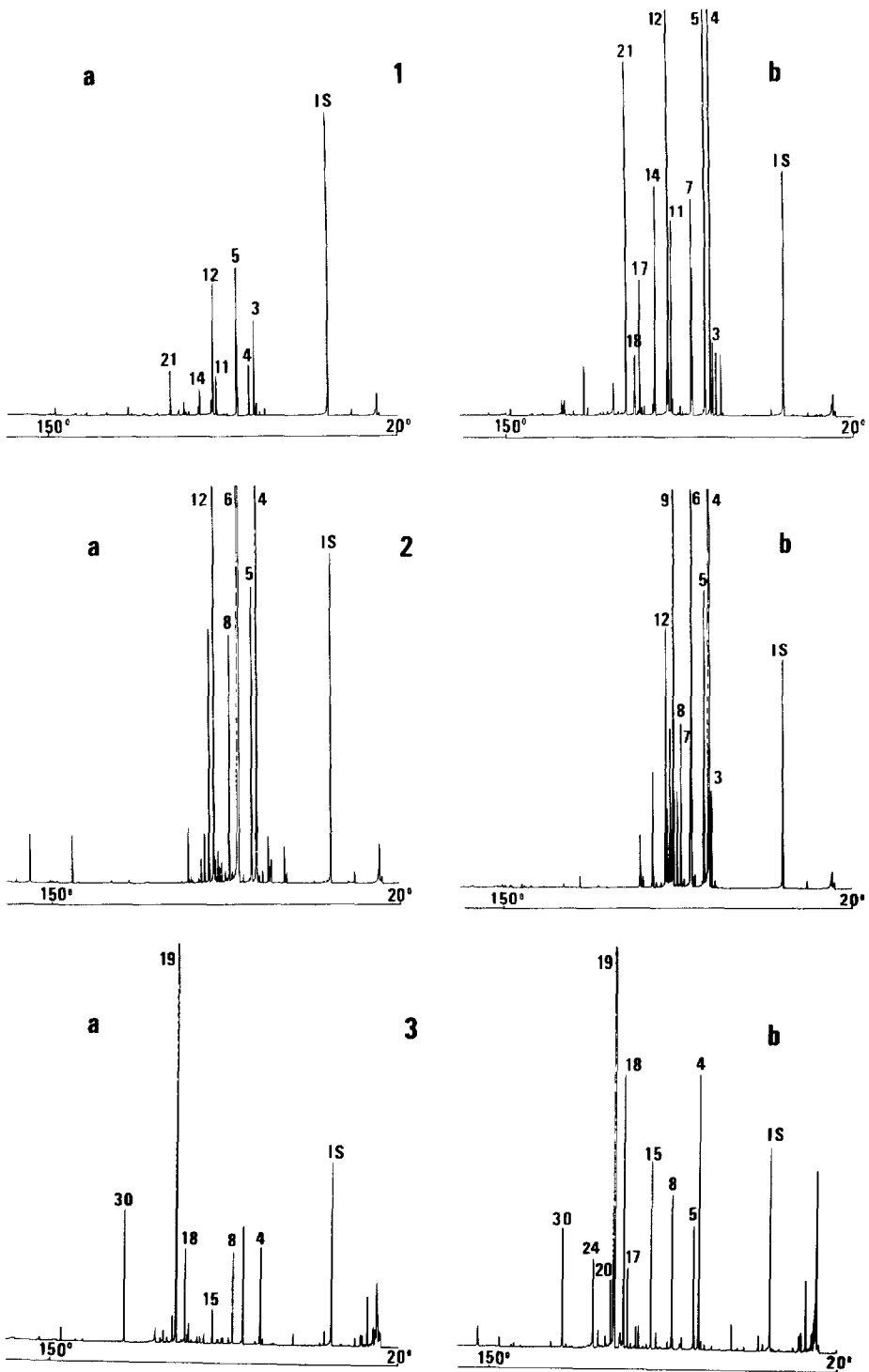
Fig. 1.
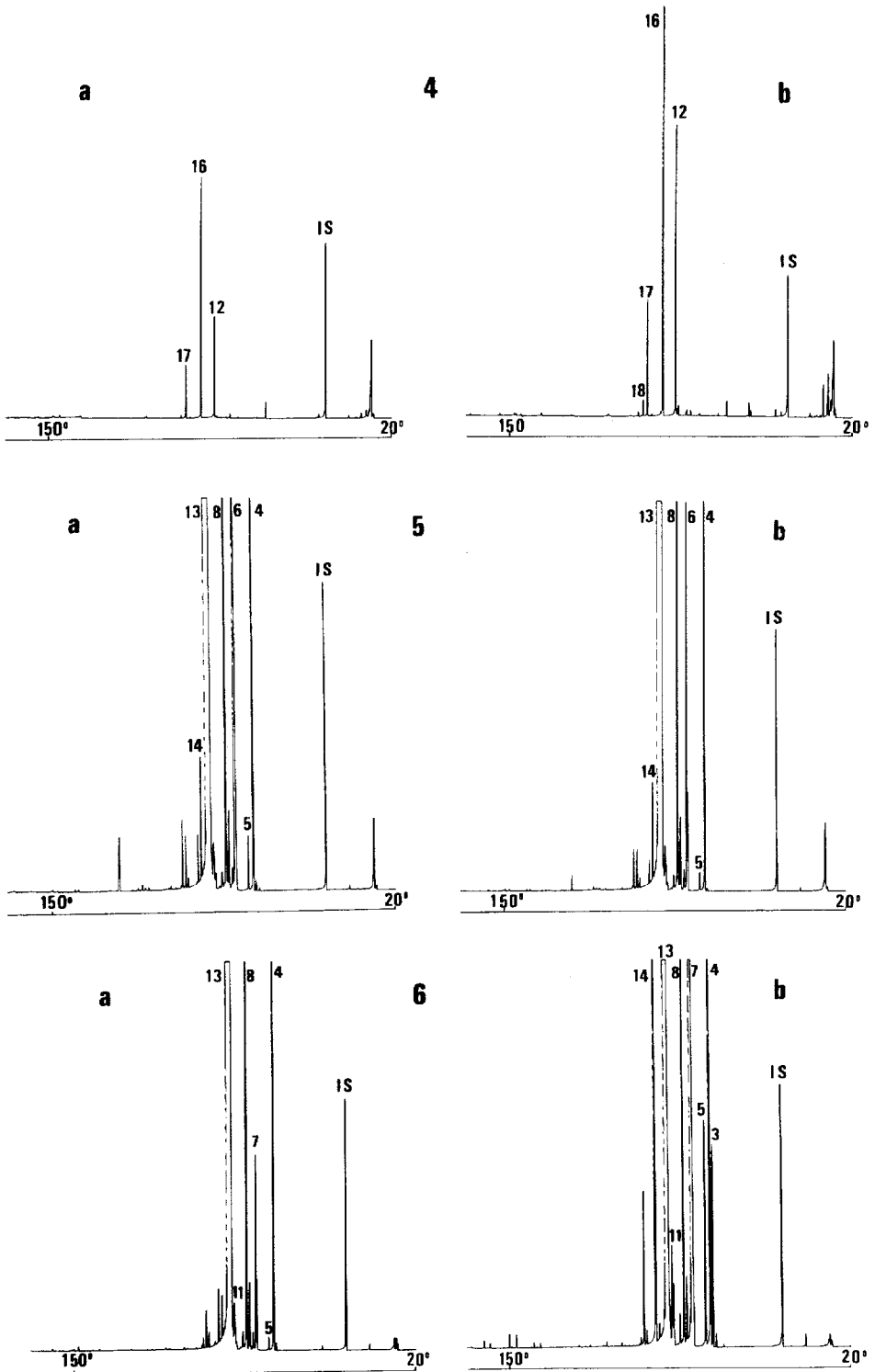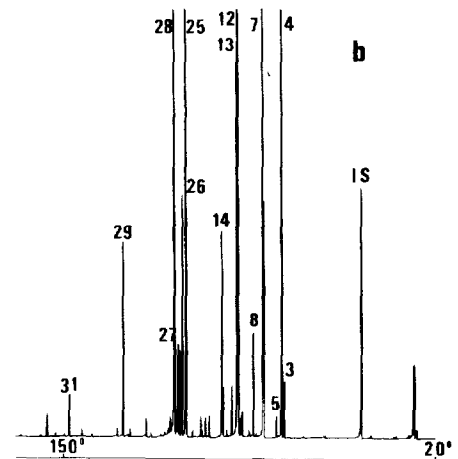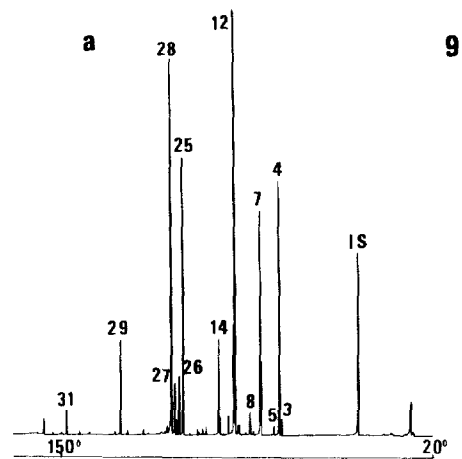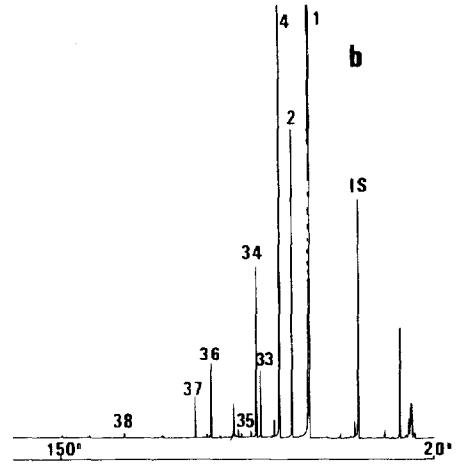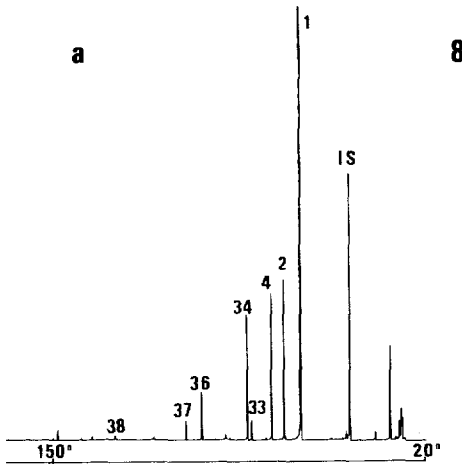
Fig. 1.

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Fig. 1. Chromatograms obtained. The numbers of the chromatograms correspond to Table I.
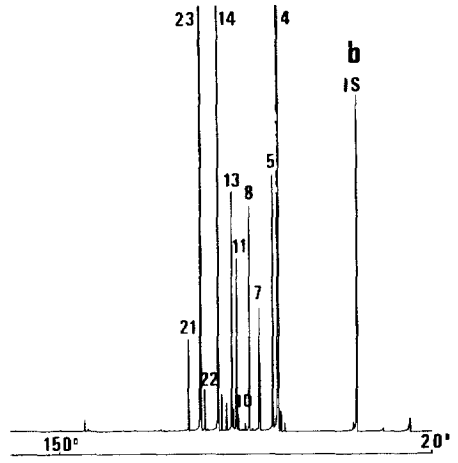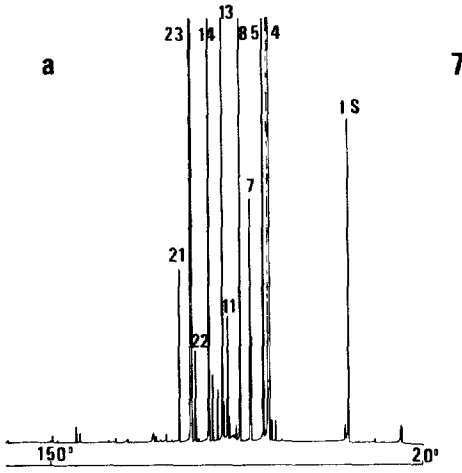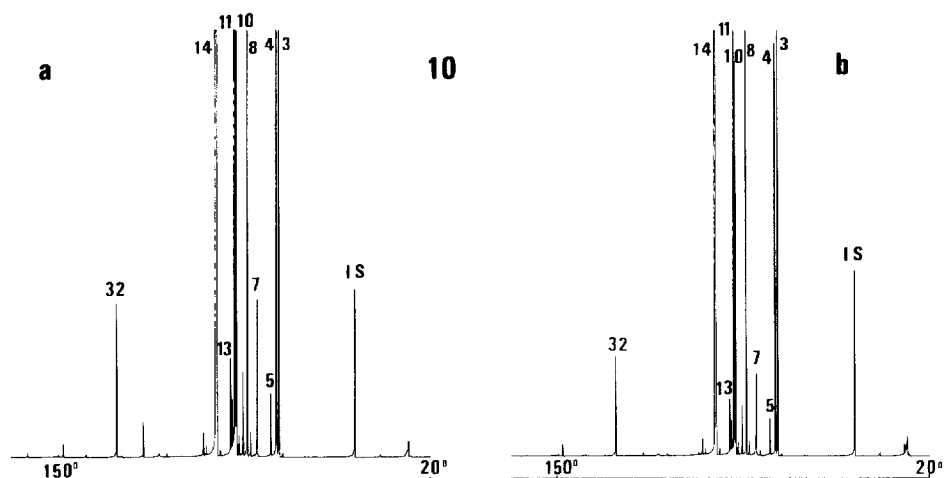
The compounds, identified by GC–mass spectrometry, are reported in Table II.

Some preliminary attempts were also made to compute the real concentration of the volatile substances present in the gaseous phase.

We used the simple multiple headspace extraction (MHE) procedure proposed by Kolb[4] for quantitative purposes. It should have allowed us to avoid the matrix effects and the influence of the partition coefficient caused by solid samples, for which no

TABLE II

COMPOUNDS IDENTIFIED IN HERBS

IS = Internal standard.

Peak number	Compound	Peak number	Compound
IS	*n*-Amyl alcohol	20	*trans*-Epoxyocimene
1	2-Methyloctane	21	Camphor
2	*n*-Nonane	22	Linalool oxide B
3	α-Thujene	23	Linalool
4	α-Pinene	24	Terpinen-4-ol
5	Camphene	25	Menthone
6	α-Phellandrene	26	Isomenthone
7	β-Pinene	27	Menthofuran
8	Myrcene	28	Menthol
9	β-Phellandrene	29	Menthylacetate
10	α-Terpinene	30	Geraniol
11	*p*-Cymene	31	Cariophyllene
12	1,8-Cineole	32	Carvacrol
13	Limonene	33	6-Methyl-5-hepten-2-one
14	γ-Terpinene	34	3-Methyl-nonane
15	Ocimene	35	*n*-Decane
16	Artemisia ketone	36	Isomdecane
17	α-Tujone	37	Undecane
18	β-Tujone	38	Tridecane
19	*cis*-Epoxyocimene		

suitable calibration standard can be prepared. Quantitative HS analysis really requires consideration of the distribution of the components under examination between the phases of the system. Unfortunately, with solid materials it is very difficult to eliminate the influence of the sample matrix on the phase equilibrium, as a result of which it is' not possible to apply the method of standard addition, commonly used in HS analysis, but which is restricted to liquid samples[5]. Of the two ways of calibration offered by Kolb's procedure, we chose the calibration by the internal standard method. Indeed, it was not possible to reproduce exactly the mixture of all volatile compounds for each herb, as the external standard method requires.

We have tested Kolb's method for one type of herb only (*Hypericum perforatum* L.), the composition of which is better known than these of the others. Our analyses are usually performed on very complex mixtures, the components of which are not yet completely identified.

In order to observe a significant decrease in the areas of the peaks, an appropriate weighed quantity of the herb was mixed with a suitable weighed amount of an inert material, such as very pure cellulose. A blank analysis was carried out on cellulose, no interfering peak was observed. The mixture was carefully ground before the HS test, and a known amount of the dust obtained was analysed.

A convenient internal standard was not easy to find. We could not use any pure compound (like hydrocarbons), either because of problems of overlapping with other substances of the herbs, or because of the difficulty in measuring a sufficiently small amount of it to be exactly compatible with the relatively small volume of the vial. Therefore, we had to use a water-soluble compound. Water is present in the herbs as moisture, and at the same time it does not disturb the course of the analysis. On the other hand, problems like those just mentioned would have arisen with any other organic solvent. An alcohol was therefore chose, the chemical and physical properties of which were found to be consistent with those of the herb and with the selected analytical conditions. We added 1 μl of a 1 % (w/w) solution of *n*-amyl alcohol in distilled water to 1 g of the mixture obtained by blending 0.5 g of St. John's wort with 19.5 g of pure cellulose. By operating in this way the real amount of the analysed herb is 25 mg, while the concentration of the internal standard corresponds to 400 ppm.

Similar tests were performed by using the internal standard on pure cellulose

TABLE III

DATA OBTAINED FROM HYDROCARBON ANALYSES

Component	Total area*	ppm**
Internal standard (*n*-amyl alcohol)	85.84	400
2-Metlyt-Octane	44.53	208
n-Nonane	13.35	62
α-Pinene	9.52	44
6-Methyl-5-hepten-2-one	2.36	11
3-Methyl-nonane	7.68	36
Isoundecane	15.53	72
Undecane	13.60	63
Tridecane	1.28	6

* Total area values drawn from the formule $\Sigma A = A_2^2 / (A_1 - A_2)$ according to Kolb[4].
** We arbitrarily assume a response factor of 1 for all the components listed.

only as a solid matrix in order to evaluate its behaviour in the absence of the other volatile compounds: no difference was observed. All trials were carried out under the conditions mentioned above.

Table III shows the data obtained from these analyses.

A suitable amount (generally 1 μl) of the internal standard solution was also added to the other spices examined, not for quantitative purposes, but in order to check its behaviour in many matrices of different composition before using it for the final quantitative analysis.

Like Kolb, we think that the additional purpose of an internal standard, *i.e.*, to compensate variations in the injected sample volume, is not important, because we use an automatic sampling system which enables us to avoid possible errors in precision and reproducibility.

CONCLUSIONS

The chromatographic differences between herbs of good quality and herbs of inferior quality, as judged by the expert tasters, are very evident and impressive. In general, the aromagrams of the best spices are richer in peaks than those of the inferior ones. Most herbs of good quality have a more complex and stronger flavour compared to related samples of poor quality. Consequently, the odour of good herbs usually gives a more intense olfactory sensation than that of the inferior ones, and the corresponding chromatograms show more peaks, which are also higher than those related to the inferior herbs. In this respect, the sensory evaluations agree with the analytical results.

The apparently contradictory data for samples 5, 7 and 10 can be explained, as mentioned above, by considering that in such cases the intensity of the flavour (and the corresponding chromatograms in which many peaks appear) is connected to less fine olfactory properties: indeed, an accurate distinction between the strength and the delicacy of a flavour is very important in the selection of the most suitable herbs for our purposes.

An accurate distinction between the strength and the delicacy of a flavour is very important in order to select the most suitable herbs for our purposes.

As far as quantitative aspects are concerned, we could agree with Kolb's results only in the case of the most volatile substances. It was impossible to find a significant decrease in the areas of the peaks for the less volatile compounds. Consequently, we could not analyse all the components present in the St. John's wort.

We can conclude, from the above results, that the HS method fits the requirements of the Quality Control Service quite well and can also be applied as a potent and useful means for the routine analysis of aromatic herbs.

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