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Article

Allergenic Fragrances Analysis in Brazilian Perfumes by Headspace Solid Phase Microextraction and Gas Chromatography-Mass Detector (HS-SPME-GC-MS)

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Perfumes are products mainly consisting of ethyl alcohol, water and fragrance. These fragrances are responsible for characterizing the pleasant and unique odor of each perfume. Among the fragrances, we highlight a group of fragrances which can cause contact allergy, leading to dermatitis. Brazilian and the European law state that when these concentrations of allergenic fragrances exceed the limit of 0.01% for non-rinse products and 0.001% for products with rinsing, the manufacturer is obliged to discriminate on its label their presence. This work aims to quantify allergic fragrances in original and Brazilian perfume using solid-phase microextraction and analyze by gas chromatography-mass detector.

Keywords: solid-phase microextraction, allergic fragrances, perfumes

Introduction

Perfumes are products of great importance in the cosmetic industry. Their essential fragrance composition is formed by ethanol and water, in which each product has a unique formulation which allows unique characteristics.[1]

Fragrances are volatile organic compounds and semi-volatile compounds which have pleasant scent characteristics. For this reason, they are used in perfumes or scented products of different purposes.[2] Some of these fragrances when used by some individuals may manifest allergic reaction when they come into direct contact with skin.[3] Clinical studies on this allergic reaction were carried out first in European Union and then Brazil to adopt more stringent legislation regarding these types of compounds.[4,5]

Some perfume components at high concentrations can cause allergic reactions. Among these compounds, stands out a group of allergic fragrances that, according to Resolution No. 3/2012 of the National Health Surveillance Agency (ANVISA, Brazil),[5] when found in excess 0.001% (m/m) and 0.01% (m/m) concentrations in products without and with rinsing, respectively, the dermocosmetic product should discriminate in its label their presence.

The gas chromatography mass spectrometry (GC-MS) is the most widely used technique in the analysis of fragrances.[6,7] Other techniques have also been recently used such as two-dimensional gas chromatography coupled with flame ionization detector,[8,9] electronic nose[10] and electrospray ionization coupled to mass spectrometer.[2,11]

The literature reports some works on analysis of allergic fragrances in various matrices such as baby bathwater,[4] fragrances,[8,9] cosmetics,[12-14] shampoo,[15] toys,[16] water types (pool and sewage),[17] indoor air[18] and fragrance oils.[19]

Due to the lack of studies in Brazil on products that can cause allergy and in order to assist the current health legislation, given the large market for skin cosmetics, we sought to investigate and quantify these compounds in perfume samples.

The aim of this work is to propose an analytical methodology for identification and quantification of allergic fragrances in Brazilian perfumes with minimal amounts of samples, using solid phase microextraction (SPME) and GC-MS.

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Experimental

Materials

In this study were investigated the standards of allergenic fragrances: 3,7-dimethyl-1,6-octadien-3-ol (linalool, 97.2%); 3,7-dimethyloct-6-en-1-ol (citronellol, 96.6%); 2-methoxy-4-prop-2-enyl phenol (eugenol, 99.6%); 2*H*-1-benzopyran-2-one (coumarin, 100%); 3,7,11-trimethyldodeca-2,6,10-trien-1-ol (farnesol, 98%); 3,7-dimethylocta-2,6-dienal (citral, 95.4%); 4-methoxybenzene ethanol (anisyl alcohol, 99.8%); 2-methoxy-4-(1-propenyl) phenol (isoeugenol, 100%); 2-(phenylmethylene)-heptanal (amylcinnamaldehyde, 98.3%); 3-phenyl phenylmethyl ester-2-propenoic acid (benzyl cinnamate, 100%); 3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one (α-isomethylionone, 100%); 3,7-dimethyl-2,6-octadien-1-ol (geraniol, 95.0%); 2-(phenylmethylene)-1-heptanol (amylcinnamic alcohol, 96.0%); 3-(4-*tert*-butylphenyl)-2-methylpropanal (lilial®, 100%); 4-(4-hydroxy-4-methylpentyl) cyclohex-3-ene-1-carbaldehyde (lyral®, 100%); 2-hydroxyphenyl-methyl ester benzoic acid (benzyl salicylate, 100%); 2-octynoic acid methyl ester (methyl 2-octynoate, 100%); 7-hydroxy-3,7-dimethyloctanal (hydroxycitronellal, 99.8%); 3-phenyl-2-propenal (cinnamaldehyde, 98.4%); 2-(phenylmethylene) octanal (hexylcinnamic aldehyde, 100%); and 3-phenyl-2-propen-1-ol (cinnamic alcohol, 94.8%) for 1000 mg L^{-1} concentration (Acunstandart, USA). SPME manual holders, 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB) fibers and vial 40 mL supplied by Supelco (Bellefonte, PA, USA), methanol grade HPLC (Merck, Brazil), NaCl PA (Vetec, Brazil) and ultrapure water obtained from a Milli-Q water purification system (Millipore, Billerica, MA, USA) were also used.

Samples

A total number of 25 fragrances were acquired: 10 dealer perfumes of authorized Brazilian brand A (PA), 5 perfumes of authorized brand B (PB) and 10 similar perfumes of brand A (PSA) found in informal trade as shown in Table 1.

Table 1. Perfume sample: brand A, brand B and similar brand A

Perfume	Type	Brand
PA 1-10	original	A
PB 1-5	original	B
PSA 1-10	similar	A

Gas chromatography-mass spectrometry (GC-MS)

The determination of the allergenic fragrance was performed on a gas chromatograph coupled with a mass detector type quadrupole GC-MS-QP2010 Plus model (Shimadzu, Japan) equipped with a DB5 column (Agilent, USA) (30 m length, 0.25 mm internal diameter and 0.25 μm film thickness, 5% phenyl and 95% polydimethylsiloxane). Helium (99.99%) was used as the carrier gas at constant flow of 1 mL min^{-1}.

The mass spectrometer conditions were set as follows: ionization mode: electron ionization (EI), 70 eV; ion source temperature 220 °C and transfer line temperature 280 °C. Quantitative analysis was performed in the selected ion monitoring (SIM) mode based on the use of one quantitative fragment and two or three qualitatives fragments. Tables 2 and 3 show the temperature program and the conditions of GC-MS and analyzed fragments. Table 3 shows the retention times of allergic fragrances and their quantitative and qualitative fragments used in SIM method.

Table 2. Chromatographic conditions

Injector temperature / °C	240
Mode	SIM
Split	1/30
Transfer line temperature / °C	280
Ion source temperature / °C	220
Temperature programming	45 °C, hold 2 min; 8 °C min^{-1} to 100 °C; 15 °C min^{-1} to 150 °C; 20 °C min^{-1} to 200 °C; 8 °C min^{-1} to 240 °C, hold 5 min. Total time 24.50 min
Cutting time / min	5.5

Table 3. SIM mode: time and fragments

time / min	Fragments
5.50-9.42	68; 67; 93; 79; 108 and 77
9.42-10.83	71; 43 and 41
10.83-18.97	41; 95; 67; 69; 44; 68; 84; 131; 103; 132; 59; 43; 71; 92; 91; 78; 164; 149; 118; 146; 89; 77; 135; 107; 150; 189; 147; 115; 129; 117; 104; 79; 93; 133; 105; 65; 138; 109 and 94
18.97-24.50	91; 131 and 77

Analysis of allergic fragrance perfume by HS-SPME-GC-MS

Previously, a multi-element stock solution in methanol of 10,000 μg L^{-1} allergenic fragrances was prepared. The following dilutions of the multi-element stock solution,

1-50 μg L^{-1}, were prepared in milli-Q solvent water. The extraction of the compounds was carried out using 10 mL of solution by adding 2 g NaCl and transferred to a 40 mL vial. The sealed vial was stirred 5 min at 100 °C. After this period, the fiber was exposed in a headspace (HS) for 20 min. Shortly after the analytes extraction, the fiber was taken and brought to the remaining gun for 15 min for complete desorption of analytes in the GC injector. This method is based on the procedure adopted by Lamas *et al.*[4] and Becerril *et al.*[17]

Method validation

Twenty one allergenic fragrances in perfumes were analyzed by HS-SPME-GC-MS. Validation parameters such as linearity, selectivity, limits of detection (LOD) and quantification (LOQ), accuracy and precision were determined according to guidelines of ANVISA-2003 and ABNT NBR 14029.[20,21] The external standard analytical curves were plotted with proper amounts of standard solutions at the concentration range of 1-50 μg L^{-1}. The significance test of the curves calibration parameters was based on a hypothesis test applying the parameter *t* test (equations 1 and 2):

$$t_{calc,A} = \frac{|A-\alpha|}{S_A} \quad (1)$$

$$t_{calc,B} = \frac{|B-\beta|}{S_B} \quad (2)$$

where A is the experimental angular coefficient; $\alpha = 1$ is the theoretical angular coefficient; $t_{calc,A}$ is the *t* value calculated for the slope; S_A is the standard deviation of the slope; B is the experimental linear coefficient; $\beta = 0$ is the theoretical linear coefficient; S_B is the standard deviation of the intercept; $t_{calc,B}$ is the *t* value calculated for the intercept.[22,23]

LOD and LOQ values were estimated in the SIM mode analysis as the lowest concentration injected. The values for LOD and LOQ were calculated taking into account the standard deviation (SD) of repetitions (n = 7) of the chromatographic analysis of the lowest point (1 μg L^{-1}) of the curve and the slope equation (AC) based on ANVISA[20] (equations 3 and 4).

$$LOD = 3.3 \times \frac{SD}{AC} \quad (3)$$

$$LOQ = 10 \times \frac{SD}{AC} \quad (4)$$

Precision of the method was evaluated by repeatability (intraday) and intermediate precision (interday) of sample solutions. The intermediate precision assays were performed in three levels of 1, 10 and 30 μg L^{-1} for three consecutive days (n = 3) and repeatability tests at a level of 30 μg L^{-1} (n = 6). The results were expressed as %RSD of the measurements. Accuracy of the method was tested with recovery experiments, performed with five replicates of blank samples spiked with 21 allergic fragrances (5, 10 and 30 μg L^{-1}) according to guidelines of ANVISA.[20]

Results and Discussion

Selectivity

In literature studies[7,9,24,25] have been shown that for allergic fragrances analysis, GC-MS in SIM mode operating system is good option to the resolution of co-elution of some compounds, however the two-dimensional chromatographic system has shown promise.

Figure 1 shows the total ion chromatogram (TIC) for all the studied compounds of the allergenic fragrance and can be observed some co-elution peaks. The co-elution occurred between citronellol (3) and anisyl alcohol (8); hydroxycitronellal (7) and citral isomer (4a); citral isomer (4b), cinnamaldehyde (6) and eugenol (10); cinnamic alcohol (9), α-isomethylioneno (13) and lyral® (16); coumarin and lilial® (12).

As can be seen in the Figure 1, the scan mode was not efficient to solve the problems of co-elution. Due to this, was chosen in order to work in SIM, because the it solved the co-elution problems and increase the sensibility, as shown in Figures 2-5.

The SIM method solves the problem of co-eluting compounds, for example, as can be seen in Figures 2 and 3, between the citral (*m/z* 84 and 94) and hydroxycitronellol (*m/z* 59). In Figures 4 and 5 can be seen between anisyl alcohol (*m/z* 138) and citronellol (*m/z* 41, 67 and 69). Table 4 shows the retention time and identification and quatitation fragments of the analytes (see Suplementary Information, Figures S1-S17).

Linearity

The calibration curves of the compounds related to allergic 21 fragrances and their correlation coefficients (R) are given in Table 5. It can be noted that all curves have an appropriate correlation coefficient value according to ANVISA.[20] The curves obtained by external standard showed good linearity as well as the results obtained by Lamas *et al.*[4] and Becerril *et al.*[17] On the other hand, Debonneville and Chaintreau[9] and Leijs *et al.*[19] investigated with success the

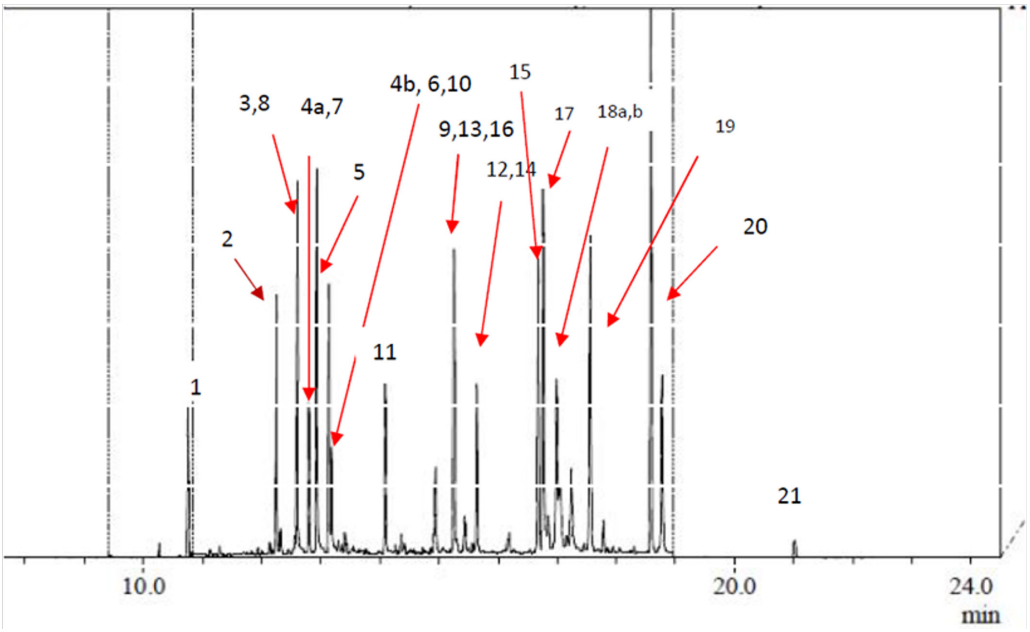

Figure 1. Total ion chromatogram (TIC) from the 21 allergic fragrances in scan mode: 1: linalool; 2: methyl 2-octynoate; 3: citronellol; 4a and 4b: citral; 5: geraniol; 6: cinnamaldehyde; 7: hydroxycitronellal; 8: anisyl alcohol; 9: cinnamic alcohol; 10: eugenol; 11: isoeugenol; 12: coumarin; 13: α-isomethylioneno; 14: lilial®; 15: amylcinnamaldehyde; 16: lyral®; 17: amylcinnamic alcohol; 18a and 18b: farnesol; 19: hexylcinnamic aldehyde; 20: benzyl salicylate; and 21: benzyl cinnamate.

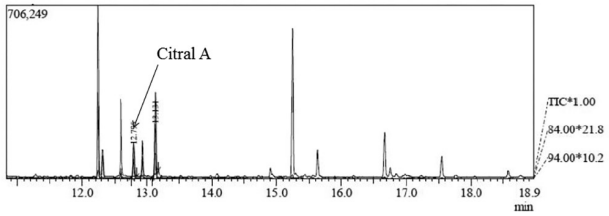

Figure 2. Chromatogram with citral fragments (*m/z* 84 and 94).

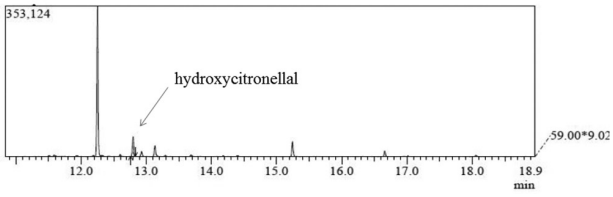

Figure 3. Chromatogram with hydroxycitronellal fragments (*m/z* 59).

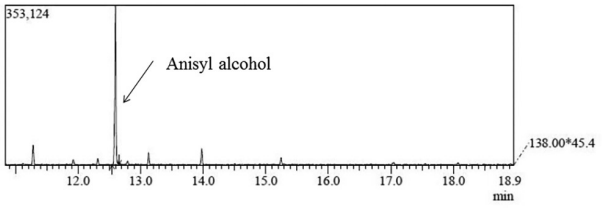

Figure 4. Chromatogram with anisyl alcohol fragments (*m/z* 138).

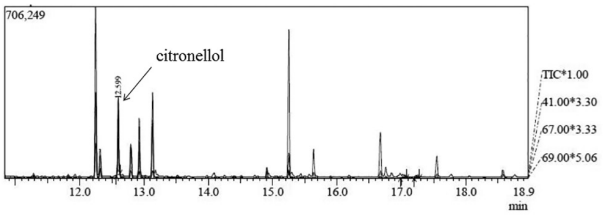

Figure 5. Chromatogram with citronellol fragments (*m/z* 41, 67 and 69).

use of two internal standards (1,4-dibromobenzene and 4,4-dibromobiphenyl) with direct injection.

Moreover, results of Table 5 showed that the technique of applying HS-SPME for those compounds did not require the use of an internal standard. However, the application of HS-SPME technique provides low limit of detection (at μg L^{-1}) for determination of these compounds within a very satisfactory linear range. The contrast was observed by studying only the headspace process as demonstrated by Sanchez *et al.*[14] which did not achieve a good linearity to the level of μg L^{-1}. These results are important from an analytical point of view, because, when working with level of concentration in μg L^{-1}, small amounts of solutes can be quantified in analytical sample, minimizing the matrix effect, in addition to preserve the integrity of the instrument.

Parameter significance testing of calibration curves

Ideally, the regression lines obtained for the calibration curve should pass through the origin, that is, the intercept of the curve passing through the point (0, 0) of the Cartesian axes. In order to evaluate the statistical significance of the regression parameters A and B, the models obtained experimentally for the calibration curves, it is useful to

compare them with theoretically expected values α and β, with α = 1 and β = 0.[2,22]

The significance test of the curves calibration parameters is based on a hypothesis test applying the parameter *t* test.

Table 4. Retention time and identification and quatitation fragments of the analytes

Compound	Retention time / min	Identification (*m/z*)	Quantification (*m/z*)
Linalool	10.75	41; 43; 71	41; 43; 71
Methyl 2-octynoate	12.25	67; 95	95
Citrolellol	12.59	41; 67; 69	41; 67; 69
Citral	12.8	84; 94	84; 94
	13.1	84; 94	84; 94
Geraniol	12.9	41; 69	41; 69
Cinnamaldehyde	13.15	77; 131	131
Hydroxycitronellal	12.75	59; 95	59
Anisyl alcohol	12.6	138; 109	138
Cinnamic alcohol	15.25	91; 92	91
Eugenol	13.2	103	103
Isoeuegenol	14.09	164	164
Coumarin	15.6	89; 118; 146	89; 118; 146
α-Isomethylioneno	15.25	107; 135; 150	107; 135; 150
Lilial®	15.64	131; 189	131; 189
Amylcinnamaldehyde	16.67	91; 115; 117	91; 115; 117
Lyral®	15.25	59; 79	79
Amylcinnamic alcohol	16.98	91; 115; 133	133
Farnesol	17.1	69; 93	69; 93
	17.25		
Hexylcinnamic aldehyde	17.55	129	129
Benzyl salicylate	18.75	65; 91	91
Benzyl cinnamate	21.00	77; 91; 131	77; 91; 131

Table 5. Calibration curves, correlation coefficient (R) of the compounds studied in a concentration range 1-50 μg L^{-1}

Compound	Linear equation	Correlation coefficient	Number of points of curve
Linalool	y = 20630x + 146779	0.995	5
Methyl 2-octynoate	y = 70166x – 67715	0.993	5
Citronellol	y = 67374x + 123450	0.995	5
Citral	y = 49185x – 67703	0.996	6
Geraniol	y = 3064.5x – 6021.9	0.996	5
Cinnamaldehyde	y = 966.41x + 1917.2	0.995	5
Hydroxycitronellal	y = 1577.1x – 1289.7	0.995	5
Anisyl alcohol	y = 18865x – 56292	0.999	5
Cinnamic alcohol	y = 1628x – 2761.9	0.997	5
Eugenol	y = 2702.7x – 2161.7	0.997	6
Isoeugenol	y = 50028x – 111773	0.996	5
Coumarin	y = 142109x – 358103	0.995	7
α-Isomethylioneno	y = 4379.2x – 7989.7	0.995	5
Lilial®	y = 128994x – 251554	0.995	5
Amylcinnamaldehyde	y = 5565x – 14563	0.995	5
Lyral®	y = 2172.9x – 947.43	0.995	5
Amylcinnamic alcohol	y = 4608.5x – 3574.6	0.996	7
Farnesol	y = 11347x – 26483	0.997	5
Hexylcinnamic aldehyde	y = 14622x – 21988	0.996	5
Benzyl salicylate	y = 70670x – 28904	0.999	6
Benzyl cinnamate	y = 1787.8x – 2566.9	0.995	5

To evaluate the statistical significance of each regression parameter, standard deviations were obtained for the slopes and intercepts of the regression equations and values of the statistical parameter *t* were calculated for each slope and intercept, according to equations 1 and 2.[22,23] The calculated value of *t* (t_{calc}) for the parameters was compared with the tabulated critical value of *t* (t_{crit}), to a confidence level of 95% and the degree of freedom (Df = N − 2) for each calibration. When t_{calc} is smaller than t_{crit} the hypothesis that the difference between the calibration parameters obtained experimentally and theoretically expected value is accepted statistically insignificant, and then the experimental calibration parameters are considered equal to the theoretical value ($\alpha = 1$ or $\beta = 0$). The results are shown in Table 6.

As can be seen in Table 6, the results of the statistical analysis of significance of the regression parameters showed that all inclinations are significant if $t_{calc,A} > t_{crit}$. The

Table 6. Results of the statistical test of significance of the parameters of the calibration curves

Compound	S_a	$t_{calc,A}$	S_b	$t_{calc,B}$	Df	$t_{Df;95\%}$	*t* test	Corrected calibration curve
Linalool	1167.4	17.67	35984.3	4.08	3	3.18	$t_{calc,A} > t_{crit}$ $t_{calc,B} > t_{crit}$	y = 20630x +146779
Methyl 2-octynoate	737.3	14.40	22904.9	2.27	3	3.18	$t_{calc,A} > t_{crit}$ $t_{calc,B} < t_{crit}$	y = 70166x
Citrolellol	3848.9	18.23	102211.3	0.66	3	3.18	$t_{calc,A} > t_{crit}$ $t_{calc,B} < t_{crit}$	y = 67374x
Citral	6.6×10^{-7}	4513155	1.4	1.17	4	2.78	$t_{calc,A} > t_{crit}$ $t_{calc,B} < t_{crit}$	y = 49185x
Geraniol	1997.1	24.63	56743.10	1.19	5	2.57	$t_{calc,A} > t_{crit}$ $t_{calc,B} < t_{crit}$	y = 3064.5x
Cinnamaldehyde	1.6×10^{-5}	193689.8	1.3	1.62	4	2.78	$t_{calc,A} > t_{crit}$ $t_{calc,B} < t_{crit}$	y = 966.41x
Hydroxycitronellal	53.0	18.20	1564.9	1.22	3	3.18	$t_{calc,A} > t_{crit}$ $t_{calc,B} < t_{crit}$	y = 1577.1x
Anisyl alcohol	7.9	198.57	256.6	2.03	3	3.18	$t_{calc,A} > t_{crit}$ $t_{calc,B} < t_{crit}$	y = 18865x
Cinnamic alcohol	849.0	22.22	21646.7	2.60	3	3.18	$t_{calc,A} > t_{crit}$ $t_{calc,B} < t_{crit}$	y = 1628x
Eugenol	76.1	21.39	2055.7	1.34	3	3.18	$t_{calc,A} > t_{crit}$ $t_{calc,B} < t_{crit}$	y = 2702.7x
Isoeuegenol	1.6×10^{-5}	183659.1	2.00	0.77	4	2.78	$t_{calc,A} > t_{crit}$ $t_{calc,B} < t_{crit}$	y = 50028x
Coumarin	3478.0	14.38	93984.1	1.19	3	3.18	$t_{calc,A} > t_{crit}$ $t_{calc,B} < t_{crit}$	y = 142109x
α-Isomethylioneno	4.2×10^{-7}	7163847	1.4	1.95	4	2.78	$t_{calc,A} > t_{crit}$ $t_{calc,B} < t_{crit}$	y = 4379.2x
Lilial®	271.8	16.10	6929.9	1.15	3	3.18	$t_{calc,A} > t_{crit}$ $t_{calc,B} < t_{crit}$	y = 128994x
Amylcinnamaldehyde	7364.2	17.52	198996.5	1.26	3	3.18	$t_{calc,A} > t_{crit}$ $t_{calc,B} < t_{crit}$	y = 5565x
Lyral®	1.3×10^{-5}	290242.3	1.37	2.08	4	2.78	$t_{calc,A} > t_{crit}$ $t_{calc,B} < t_{crit}$	y = 2172.9x
Amylcinnamic alcohol	81.6	26.61	2399.2	0.39	5	2.57	$t_{calc,A} > t_{crit}$ $t_{calc,B} > t_{crit}$	y = 4608.5x
Farnesol	7.6×10^{-6}	391873.2	1.04	0.86	4	2.78	$t_{calc,A} > t_{crit}$ $t_{calc,B} < t_{crit}$	y = 11347x
Hexylcinnamic aldehyde	4.0×10^{-6}	758154.8	1.27	1.98	4	2.78	$t_{calc,A} > t_{crit}$ $t_{calc,B} < t_{crit}$	y = 14622x
Benzyl salicylate	3.4×10^{-6}	891759.7	1.33	1.47	4	2.78	$t_{calc,A} > t_{crit}$ $t_{calc,B} < t_{crit}$	y = 70670x
Benzyl cinnamate	101.7	17.57	2812.3	0.91	3	3.18	$t_{calc,A} > t_{crit}$ $t_{calc,B} < t_{crit}$	y = 1787.8x

S_a: standard deviation of the slope; S_b: standard deviation of the intercept; $t_{calc,A}$: *t* value calculated for the slope; $t_{calc,B}$: *t* value calculated for the intercept; Df: degree of freedom; $t_{Df;95\%}$: Student's *t*-test with confidence level of 95%.

intercept, with the exception of the compound of linalool curve, is not significant, considering that $t_{calc,B} < t_{crit}$ and was considered as statistically equal to β, which has a value of zero reference. Therefore, the equations of the curves applied to the calculations of the analytes concentrations are the ones shown in Table 6, with a 95% confidence level. Among the tested compounds, the only compound which shows the statistically significant linear coefficient is linalool.

Limits of detection (LOD) and quantification (LOQ)

The results for the limits of detection (LOD) and quantification (LOQ) are shown in Table 7. The LOD and LOQ values calculated in this work took into account the standard deviation (SD) of repetitions (n = 7) of the chromatographic analysis of the lowest point (1 μg L^{-1}) of the curve and the slope equation (AC) based on ANVISA.[20]

Table 7. Limit of detection (LOD) and limit of quantification (LOQ) of allergic fragrances

Compound	LOD / (μg L^{-1})	LOQ / (μg L^{-1})
Linalool	0.074	0.247
Methyl 2-octynoate	0.043	0.142
Citronellol	0.008	0.027
Citral	0.017	0.057
Geraniol	0.040	0.135
Cinnamaldehyde	0.245	0.878
Hydroxycitronellal	0.239	0.797
Anisyl alcohol	0.712	0.234
Cinnamic alcohol	0.043	0.143
Eugenol	0.270	0.900
Isoeugenol	0.064	0.215
Coumarin	0.202	0.674
α-Isomethylioneno	0.201	0.670
Lilial®	0.101	0.338
Amylcinnamaldehyde	0.005	0.017
Lyral®	0.243	0.810
Amylcinnamic alcohol	0.252	0.840
Farnesol	0.074	0.248
Hexylcinnamic aldehyde	0.211	0.702
Benzyl salicylate	0.271	0.904
Benzyl cinnamate	0.210	0.701

Precision

The analysis of the coefficient of variation values (CV%), repeatability and intermediate precision showed values ranging from 2.6 to 19.2%, as seen in Table 8. According to ANVISA, an acceptable value is around 5%; however, because of the magnitude of these compounds in the sample to the level 1 μg L^{-1}, other references[20,21,26] also accept a variation coefficient value of up to 20%. Therefore, our results can be considered acceptable, because, according to the norm of ABNT NBR 14029,[21] which stipulates that for a chemical analysis of the magnitude 10-100 μg L^{-1}, the value of coefficient of variation between 23 to 32% is accepted, although this rule is for pesticide analysis at this level of concentration.

Our results of intermediate precision presented values lower than 20%, however the literature reports results lower than 10%. However, values obtained in this study (1, 10 and 30 μg L^{-1}) do not follow a quantitative standard for intermediate precision as expected, but it was also observed in other studies.[17] Analyzing the CV% for the repeatability study in Table 8, it can be seen that the values were above 15%. These values are acceptable according to the standard 14029 of ABNT-NBR.[21] However, when compared to literature data,[17] it is noted that the CV values obtained are less than 10%, but these results are for concentration of 20 μg L^{-1}. Therefore, as in this work the concentration of the compounds studied was 30 μg L^{-1}, then the difference in the CV values may be due to this fact.

Recovery

Recovery results were satisfactory for most of the 21 compounds analyzed according to ANVISA standard.[20] Looking at Table 9, the compounds which showed poor recovery were cinnamic alcohol (10 and 30 μg L^{-1}) and lilial (30 μg L^{-1}), but considering the RSD these results are consistent with current regulations of 70-120%. The comparison of the recovery values obtained with literature data[4,17] indicates that our values are lower. This can be explained by the complex nature of the perfume composition, which can interfere with the recovery rate of the studied compounds.

Analysis of perfumes

From the results of the analyzes of perfumes (Table 10), it can be observed the presence of some of these fragrances in high concentrations that can cause allergy and thus they need to be informed in the products' labels for consumers' knowledge and meet the current legislation. Among the fragrances studied it was observed that the original perfumes (brands A and B) present allergenic fragrances in high concentrations for all the range of molar weight of the compounds. However, for the similar perfumes was not observed for fragrances with high molar weight, such as benzyl cinnamate and hexylcinnamic aldehyde. This can

Table 8. Intermediate precision and repeatability of allergic fragrances

Compound	Intermediate precision			Repeatability
	1 μg L^{-1} (n = 3)	10 μg L^{-1} (n = 3)	30 μg L^{-1} (n = 3)	30 μg L^{-1} (n = 6)
Linalool	4.4 (6.4)[a]	2.6 (12)[b]	19.3 (4.6)[c]	16.3 (1)[d]
Methyl 2-octynoate	9.7 (8.6)[a]	6.9 (12)[b]	13.6 (8.6)[c]	8.6 (1)[d]
Citronellol	1.6 (8.7)[a]	3.1 (7.9)[b]	10.2 (6.5)[c]	14.0 (9.6)[d]
Citral	8.2 (1.2)[a]	6.2 (3.4)[b]	10.9 (2.9)[c]	15.6 (0.8)[d]
Geraniol	14.9 (6.6)[a]	8.3 (5.3)[b]	13.3 (2.5)[c]	13.9 (0.6)[d]
Cinnamaldehyde	13.3 (7.7)[a]	5.6 (12)[b]	7.5 (4.3)[c]	13.0 (1)[d]
Hydroxycitronellal	15.3 (4.3)[a]	4.1 (11)[b]	16.4 (3.7)[c]	15.2 (3.6)[d]
Anisyl alcohol	15.8 (3.6)[a]	11.9 (11)[b]	18.4 (6.0)[c]	15.4 (7.1)[d]
Cinnamic alcohol	4.4 (5.3)[a]	5.7 (5.4)[b]	10.9 (7.5)[c]	16.2 (5.9)[d]
Eugenol	15.4 (6.5)[a]	10.7 (6.6)[b]	4.8 (3.9)[c]	15.7 (2.8)[d]
Isoeugenol	6.6 (6.3)[a]	13.9 (0.6)[b]	3.7 (2.4)[c]	6.7 (3)[d]
Coumarin	6.5 (7.1)[a]	13.5 (7.7)[b]	9.8 (3.7)[c]	17.5 (4.3)[d]
α-Isomethylioneno	3.1 (7.0)[a]	5.4 (2.5)[b]	11.2 (0.8)[c]	13.8 (0.9)[d]
Lilial®	3.0 (4.8)[a]	11.3 (4.8)[b]	8.7 (3.0)[c]	16.3 (1.6)[d]
Amylcinnamaldehyde	9.5 (4.6)[a]	16.9 (8.9)[b]	6.3 (3.7)[c]	19.7 (2.5)[d]
Lyral®	18.0 (11)[a]	7.4 (17)[b]	12.5 (3.2)[c]	8.9 (1.4)[d]
Amylcinnamic alcohol	14.3 (5.9)[a]	11.4 (5.5)[b]	15.5 (4.3)[c]	14.4 (0.8)[d]
Farnesol	10.2 (7.1)[a]	11.2 (6.3)[b]	13.9 (1.9)[c]	16.8 (1.4)[d]
Hexylcinnamic aldehyde	3.6 (4.0)[a]	5.9 (8.8)[b]	13.2 (3.3)[c]	16.1 (2.5)[d]
Benzyl salicylate	16.8 (11)[a]	17.9 (3.2)[b]	7.3 (9.2)[c]	15.6 (7.6)[d]
Benzyl cinnamate	13.6 (12)[a]	12.0 (15)[b]	12.8 (5.3)[c]	19.2 (6.8)[d]

[a]Intermediate precision 5 μg L^{-1} (N = 5);[17] [b]intermediate precision 10 μg L^{-1} (N = 5);[17] [c]intermediate precision 20 μg L^{-1} (N = 5);[17] [d]repeatability 20 μg L^{-1} (N = 3).[17]

Table 9. Recovery of allergic fragrances

Compound	Recovery (RSD) / %		
	5 μg L^{-1} (n = 3)	10 μg L^{-1} (n = 3)	30 μg L^{-1} (n = 3)
Linallol	70 ± 13.1	111 ± 8.3	75 ± 8.4
Methyl 2-octynoate	72 ± 11.3	88 ± 4.9	92 ± 5.7
Citronellol	90 ± 5.8	80 ± 11.5	107 ± 6.3
Citral	70 ± 6.7	88 ± 7.4	98 ± 9.7
Geraniol	86 ± 8.4	73 ± 13.6	76 ± 6.2
Cinnamaldehyde	83 ± 7.9	77 ± 10.5	94 ± 8.2
Hydroxycitronellal	69 ± 7.5	87 ± 9.1	105 ± 6.6
Anisyl alcohol	78 ± 12.1	86 ± 7.2	82 ± 12.4
Ainnamic alcohol	86 ± 8.7	59 ± 4.5	66 ± 10.3
Eugenol	78 ± 9.5	76 ±5.7	103 ± 8.1
Isoeugenol	89 ± 13.2	82 ± 4.8	110 ± 4.8
Coumarin	103 ± 7.1	94 ± 6.9	88 ± 12.9
α-Isomethylioneno	73 ± 4.9	113 ± 4.2	109 ± 8.4
Lilial®	87 ± 7.7	66 ± 11.2	89 ± 3.9
Amylcinnamaldehyde	86 ± 9.4	114 ± 8.2	101 ± 11.9
Lyral®	72 ± 6.9	115 ± 9.0	112 ± 7.9
Amylcinnamic alcohol	81 ± 4.7	90 ± 6.6	87 ± 7.4
Farnesol	80 ± 7.3	111 ± 8.9	78 ± 4.9
Hexylcinnamic aldehyde	92 ± 5.9	85 ± 5.4	98 ± 9.6
Benzyl salicylate	71 ± 8.7	93 ± 8.5	102 ± 5.3
Benzyl cinnamate	103 ± 6.8	99 ± 10.1	88 ± 8.1

Table 10. Result analysis fragrances in original perfumes and similar

Sample	Concentration / (mg L^{-1})											
	Linalool	Methyl 2-octynoate	Citronellol	Citral	Geraniol	Cinnamaldehyde	Hydroxy citronellal	Anisyl alcohol	Cinnamic alcohol	Eugenol	Isoeugenol	Coumarin
PA1	< LOD	< LOD	< LOD	< LOD	58.9	< LOD	31.5	< LOD	16.2	22.2	28.2	< LOD
PA2	50.2	< LOD	8.6	19.8	60.5	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	21
PA3	30.9	< LOD	< LOD	12.3	82.5	< LOD	15.2	< LOD	< LOD	13.7	25.6	47.9
PA4	8.8	< LOD	7.4	< LOD	11	< LOD	9.0	< LOD	< LOD	19.1	< LOD	13.5
PA5	41.6	< LOD	< LOD	9.9	52.1	8.9	< LOD	< LOD	9.0	182.5	24.8	81.1
PA6	42.1	< LOD	< LOD	42.1	77.8	< LOD	32.9	< LOD	< LOD	< LOD	< LOD	42.9
PA7	14.1	< LOD	9.1	< LOD	1.1	2.1	25.9	< LOD	< LOD	< LOD	< LOD	< LOD
PA8	11.5	< LOD	< LOD	19	42.4	1.9	13	< LOD	< LOD	9.1	66.5	10.3
PA9	< LOD	< LOD	4.7	1.4	22	38.7	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
PA10	12.7	< LOD	5.5	7.0	22.2	1.3	34.2	< LOD	< LOD	< LOD	< LOD	< LOD
PB1	46.4	< LOD	8.7	10	77.4	1.7	11.7	< LOD	< LOD	< LOD	< LOD	< LOD
PB2	< LOD	< LOD	19.8	6.0	46.5	< LOD	7.0	< LOD	12.3	< LOD	< LOD	41.7
PB3	35.4	< LOD	13.7	1.1	21.3	< LOD	< LOD	< LOD	< LOD	11	< LOD	< LOD
PB4	30.0	< LOD	11.2	8.3	31	6.3	9.0	< LOD	< LOD	10.4	< LOD	< LOD
PB5	36.3	< LOD	< LOD	17.2	86.5	1.6	14.1	< LOD	13.5	19	< LOD	34.5
PSA1	31.4	< LOD	6.7	8.2	56.5	< LOD	< LOD	< LOD	< LOD	1.2	1.4	< LOD
PSA2	14.7	< LOD	8.0	3.5	28.2	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
PSA3	< LOD	< LOD	1.0	1.4	19.2	< LOD	1.3	< LOD	< LOD	< LOD	1.3	5.6
PSA4	< LOD	< LOD	10.6	< LOD	23.4	< LOD	< LOD	< LOD	< LOD	1.9	1.1	0.0
PSA5	17.7	< LOD	13.7	< LOD	2.0	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	1.8
PSA6	2.2	< LOD	< LOD	< LOD	46.8	< LOD	10.6	< LOD	< LOD	8.3	< LOD	< LOD
PSA7	17.6	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
PSA8	34.9	< LOD	< LOD	7.2	34.0	< LOD	< LOD	< LOD	< LOD	1.6	< LOD	< LOD
PSA9	12.6	< LOD	< LOD	< LOD	19.2	< LOD	2.6	< LOD	< LOD	2.3	< LOD	< LOD
PSA10	8.4	< LOD	6.6	< LOD	28.8	< LOD	< LOD	< LOD	< LOD	1.7	< LOD	< LOD

Sample	Concentration / (mg L^{-1})								
	α-Isomethyl ioneno	Lilial®	Amylcinnamaldehyde	Lyral®	Amylcinnamic alcohol	Farnesol	Hexylcinnamic aldehyde	Benzyl salicylate	Benzyl cinnamate
PA1	36.1	164.7	< LOD	31.0	< LOD	30.7	< LOD	< LOD	< LOD
PA2	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	39.1	< LOD	< LOD
PA3	< LOD	128.5	< LOD	16.6	< LOD	31.1	< LOD	7.1	< LOD
PA4	< LOD	< LOD	< LOD	7.0	< LOD	8.2	12.6	1.3	< LOD
PA5	32.7	162.8	< LOD	29.9	< LOD	< LOD	< LOD	< LOD	< LOD
PA6	1.5	76.1	< LOD	< LOD	22.1	< LOD	16.4	2.3	< LOD
PA7	< LOD	43.5	< LOD	< LOD	2.4	34.6	< LOD	37.4	< LOD
PA8	< LOD	15.0	< LOD	< LOD	< LOD	< LOD	2.6	< LOD	< LOD
PA9	< LOD	9.4	< LOD	12.9	14.9	< LOD	3.2	55.7	< LOD
PA10	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
PB1	13.1	106.6	< LOD	11.3	8.3	< LOD	< LOD	190	< LOD
PB2	17.4	51.2	< LOD	26.5	14.1	< LOD	1.6	14.2	< LOD
PB3	9.4	48.8	< LOD	< LOD	9.1	< LOD	< LOD	3.3	< LOD
PB4	11.2	46.4	< LOD	12.6	7.5	< LOD	< LOD	30.1	< LOD
PB5	< LOD	8.7	< LOD	< LOD	12.5	< LOD	12.8	< LOD	< LOD
PSA1	3.0	3.5	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
PSA2	1.5	2.6	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
PSA3	< LOD	8.8	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
PSA4	2.3	22.7	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
PSA5	< LOD	9.6	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
PSA6	< LOD	6.9	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
PSA7	< LOD	1.9	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
PSA8	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
PSA9	2.8	4.7	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
PSA10	< LOD	4.7	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD

be explained due to the low capacity of scent fixation of similars perfumes when compared to originals.

Conclusion

This technique can be used for analysis of allergic fragrance perfume using small amount of sample for analysis. This study may be a help to ANVISA in the control of allergic fragrances in perfumes. The results indicate that the similar perfumes do not present allergenic fragrances with high molecular weight, which influences an inferior scent fixation when compared to the original perfumes.

Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br as PDF file.

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