Process to Formulate Hypoallergenic Jasmin Oil

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As a major cosmetic company, we cannot put our products on the market without taking their safety into consideration. These days fragrance materials have been considered suspect in connection with cosmetic contact dermatitis. Our efforts to isolate and identify allergens contained in jasmin oil and to develop hypoallergenic jasmin oil permitted us to formulate our products with safe fragrance materials.

Even though jasmin oil is an extremely important natural oil used to formulate perfumes, cosmetics and toiletries, this natural oil is one of those materials that has been reported by dermatologists to cause positive reactions in patients with cosmetic contact dermatitis. Its allergenicity has become a great concern of cosmetic scientists.

Contact Allergenicity of Jasmin Oil

Table I is an example of clinical studies related to the contact allergenicity of jasmin oil. Dr. Sugai, a Japanese dermatologist, reported a list of Japanese standard allergens in the order of the incidence of positive reactions when tested with patients with contact dermatitis during the period of September 1973 to December 1981.[1] Eighty-one out of 1346 patients showed positive reaction at 1% concentration of jasmin oil.

Positive reactions to jasmin oil were reported in the United States as well.[2] The North American Contact Dermatitis Group reported the patch test results of important perfume materials in patients (Table II). The incidence of positive reactions to absolute jasmin oil ranked second among those materials tested.

In order to evaluate contact allergenicity of jasmin oil, the guinea pig maximization test developed by Drs. Magnusson and Kligman was employed.[3,4] Figure 1 briefly explains the procedure of this test method.

A pair of three samples were injected on the

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Table I. The Incidence of the positive reactions with standard allergens (from September 1973 to December 1981)

No.	Allergens	Concentration(%)	Vehicles	Positive	Tested	%
1	Co^{++}	1	Pet.	193	2236	8.6
2	Cr^{6+}	0.5	Pet.	163	2344	7.0
3	Ni^{++}	2.5	Pet.	135	1990	6.8
4	Wood tars	5	Pet.	44	672	6.5
5	Hg^{++}	0.05	Water	61	936	6.5
6	Benzyl salicylate	5 and 2	Pet.	77	1255	6.1
7	Jasmin absolute	1	Pet.	81	1346	6.0
8	Formalin	2	Water	147	2496	5.9
9	Ylang-ylang oil	2	Pet.	80	1368	5.8
10	Quinolin yellow SS	5 and 0.5	PEG or Pet.	36	648	5.6
11	Sudan I	0.1	Pet.	30	577	5.2
12	Hydroxycitronellal	10 and 5	Pet.	71	1401	5.1
13	p-Phenilenediamine	1	Pet.	95	1879	5.1
14	Fradiomycin	20	Pet.	85	1684	5.0
15	Cinnamic aldehyde	2	Pet.	51	1178	4.3

Vehicle: Pet. = Petrolatum, PEG = Polyethyleneglycol by Dr. Sugai (1982)

nuchal region; these are emulsified Freund's Complete Adjuvant, test substance, and test substance in Freund's Complete Adjuvant.

One week following the injections, sodium lauryl sulfate in petrolatum was applied to enhance the penetration of the test substance. On the next day, the test material was applied occlusively for 48 hours. After a two week rest period, the challenge application was performed on the flank of animals clipped free of hair. The reaction was read at 24 and 48 hours after challenge application with respect to erythema and edema.

We employed the criteria of the skin responses shown in Table III. Fractional response, which indicates the incidence of positive reactions per number of animals tested, and mean response, which represents the intensity of the reaction, were calculated by the formula shown in this table.

The results of the allergenicity of five hexane-type absolute natural jasmin oils are summarized in Table IV. Induction concentration was 10% and challenge was performed by topical application with open patch test technique. All of them were demonstrated to possess not only strong allergenicity but cross reactivity as well. An attempt was made to identify the allergens from these samples.

Isolation and Identification of Contact Allergens

Jasmin oil, which showed strong allergenicity in the guinea pig maximization test, was distilled to separate a distillate fraction from the residue under relatively mild conditions. Strong allergic

Table II. Patch Test Results with a Perfume Series

Perfume Allergens		No. of Patients Tested	% Reactivity
1. Jasmin synthetic	10%	183	15.3
2. Jasmin absolute	10%	185	10.8
3. Coumarin	5%	183	5.4
4. Isoeugenol	2%	273	5.1
5. Eugenol	2%	168	4.2
6. Cinnamic aldehyde	?	202	3.4
7. Benzyl salicylate	2%	183	2.1
8. Methyl salicylate	2%	183	1.6
9. Musk ambrette	5%	183	1.6
10. Benzyl benzoate	2%	198	0
11. Costus root oil	0.1%	148	0

E. J. Rudner (1977) Contact Dermatitis 3:208.9

Table III. Scale for Scoring Skin Reactions

(1) Erythema formation
- No erythema 0
- Very slight erythema (barely perceptible) . 1
- Well-defined erythema 2
- Moderate to severe erythema 3
- Severe erythema (beet redness) to slight eschar formation (injuries in depth). . . 4

(2) Edema formation
- No edema 0
- Slight edema. 1
- Moderate edema. 2
- Severe edema (raised more than 1 mm and extending beyond area of exposure). . . . 3

Overall maximum score. . 7

$$\text{Mean response} = \frac{\sum_{1}^{n} [(1) + (2)]}{\text{Total number of animals}}$$

Table IV. Allergenicity of Five Jasmin Oils

Sample Challenged on Animal	Challenge Concentration (%)	Sample Induced to Animal					Control
		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	
Sample 1	10	10/10 (3.8)*	10/10 (1.3)	10/10 (1.3)	9/10 (1.1)	10/10 (2.4)	1/10 (0.1)
Sample 2	10	10/10 (1.3)	10/10 (3.2)	10/10 (2.6)	10/10 (3.0)	10/10 (3.9)	0/10 (0)
Sample 3	10	10/10 (1.3)	10/10 (1.8)	10/10 (2.7)	10/10 (2.6)	10/10 (3.1)	0/10 (0)
Sample 4	10	10/10 (1.3)	10/10 (1.8)	10/10 (2.1)	10/10 (2.4)	10/10 (2.2)	0/10 (0)
Sample 5	10	10/10 (1.0)	10/10 (1.7)	10/10 (1.9)	10/10 (1.2)	10/10 (2.6)	0/10 (0)

* All results are shown as "Number of positively reacted animals/Number of animals tested (Average score of allergenic reaction)".

reactions were observed in the group treated with the residue fraction, while no reaction was observed with the distillate fraction (figure 2).

The residue fraction was further fractionated to isolate contact allergens.

Such methods as molecular distillation, second ordinary distillation and gel permeation chromatography were employed. The fractions obtained at each step of the fractionation procedure were evaluated for their allergenicity (figure 3).

It turned out that allergenic materials were concentrated in Fractions E and F (Table V). In order to determine the chemical structures of E and F, such analytical procedures as FT-IR (Fourier Transform Infrared: figure 4), proton- and carbon 13-NMR (Nuclear Magnetic Resonance: figures 5 and 6) were utilized. From the analysis of these data coupled with GC analysis of trimethylsilyl derivatives of E and F, E was identified as coniferyl benzoate and F as coniferyl acetate, whole chemical formulae are shown in figure 7.

Both coniferyl benzoate and coniferyl acetate were synthesized from coniferyl alcohol. Synthesized, these compounds proved to be allergenic (Table VI).

Process to Formulate Hypoallergenic Jasmin Oil

The process to remove the allergenic substances from the natural jasmin oil and to formulate the hypoallergenic jasmin oil (figure 8) follows.

The first ordinary distillation was performed under relatively mild conditions. It was found that the main allergens such as coniferyl benzoate and coniferyl acetate were concentrated in the residue of distillation I (DI-R). We attempted to remove these allergens from the residue. Even though the ordinary distillation procedure is excellent to separate a mixture of compounds, the decomposition of the substances with high boiling point results in the generation of undesirable odor. The residue (DI-R) fraction containing compounds with high boiling point, which are vulnerable to heat decomposition, was subjected to molecular distillation at a temperature of not more than 110°C.

The first distillate (MDI-1), which had weak allergenicity, was further distilled to obtain the nonallergenic fraction DII-1. It was confirmed by the GPC (gel permeation chromatography) and GC-MF (gas chromatograph-mass fragment graph) analysis that the DII-1 fraction did not contain either coniferyl benzoate or coniferyl acetate.

Since the residue of molecular distillation (MDI-R) contains important components characteristic of the note of jasmin oil, an adsorbent column chromatography was utilized to eliminate the allergenic compounds from this

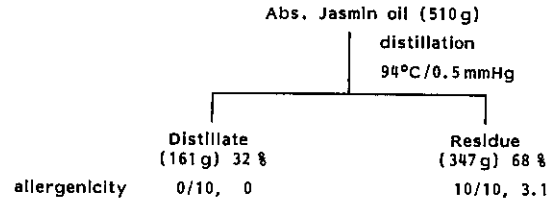
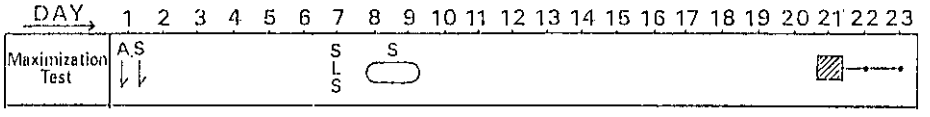
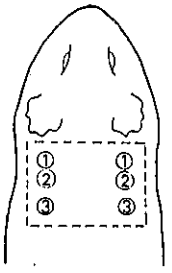


Figure 2. Allergenicity of fractions following distillation



A ; Adjuvant, S ; Sample, ↓ ; Intradermal injection, ⟷ ; Observation,
⬭ ; Closed patch, ▨ ; Challenge, SLS ; Topical application of 10% SLS



① : 0.1 ml of emulsified adjuvant
② : 0.1 ml of test substance
③ : 0.1 ml of the test agent emulsified in the adjuvant

Figure 1. Procedure of guinea pig maximization test

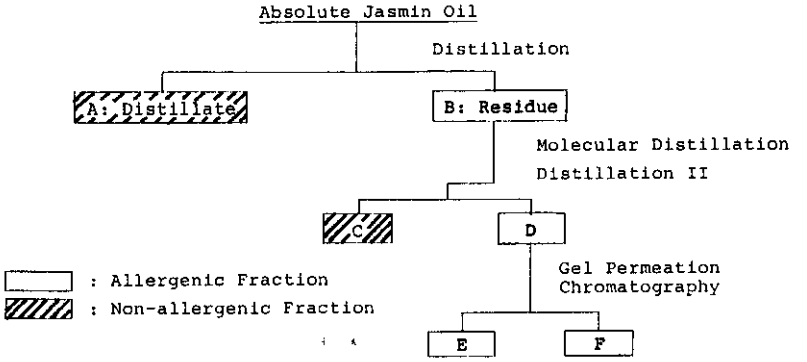


Figure 3. Procedure to isolate and identify allergens

Table V. Allergenicity of Fractions E and F

Challenge Sample	Concentration (% in Acetone)	Treated Group	Control Group	Contact Allergenicity
Fraction E	0.2	10/10 (2.2)	0/10	Yes
Fraction F	0.2	10/10 (2.6)	0/10	Yes

Table VI. Contact Allergenicity of Coniferyl Benzoate and Coniferyl Acetate

Challenge Sample	Challenge test Concentration % in Acetone	Sensitized Animal Group	Control Animal Group	Allergenicity
Coniferyl benzoate	1	5/5 2.8	0/5 0	Yes
Coniferyl acetate	1	5/5 2.8	0/5 0	Yes

Table VII. Hypo-Allergenic Jasmin Oil: Example 1

	%
DI-1	32.0
DII-1	15.0
Adsorbent treated portion (Effluence)	18.0
Methyl dihydro jasmonate	3.0
cis-Jasmone	1.0
alpha-Hexyl cinnamic aldehyde	1.0
Diisobutyl adipate	5.0
Propylene glycol dibenzoate	8.5
Trimethylol propane triisostearate	8.0
Glucam P20	8.2
Jasmin base S	0.3
	100.0

fraction. The effluent obtained with such non-polar solvent as n-pentane or n-hexane did not exhibit allergenicity.

Thirty-two percent distillate of distillation I, 15% distillate of distillation II and 18% adsorbent column effluent from MDI-R were combined and used as major components to formulate hypoallergenic jasmin oil. The yield was 65% in this case.

Hypoallergenic jasmin oil obtained in this way turned out to be inferior to natural jasmin oil with respect to the harmony and the duration of the note. In the use of such compounds as perfume fixatives, a small amount of synthetic or natural oils is necessary to make hypoallergenic jasmin oil have an excellent odor.

Table VII is an example of the formula for hypoallergenic jasmin oil, composed of hypoallergenic jasmin portions, fixatives and so on. The hypoallergenic jasmin oil presented here gives an excellent odor, similar to that of natural jasmin oil.

Allergenicity of Hypoallergenic Jasmin Oil

The allergenicity of hypoallergenic jasmin oil formulated this way was very low compared with that of absolute jasmin oil when evaluated with guinea pig maximization test (Table VIII).

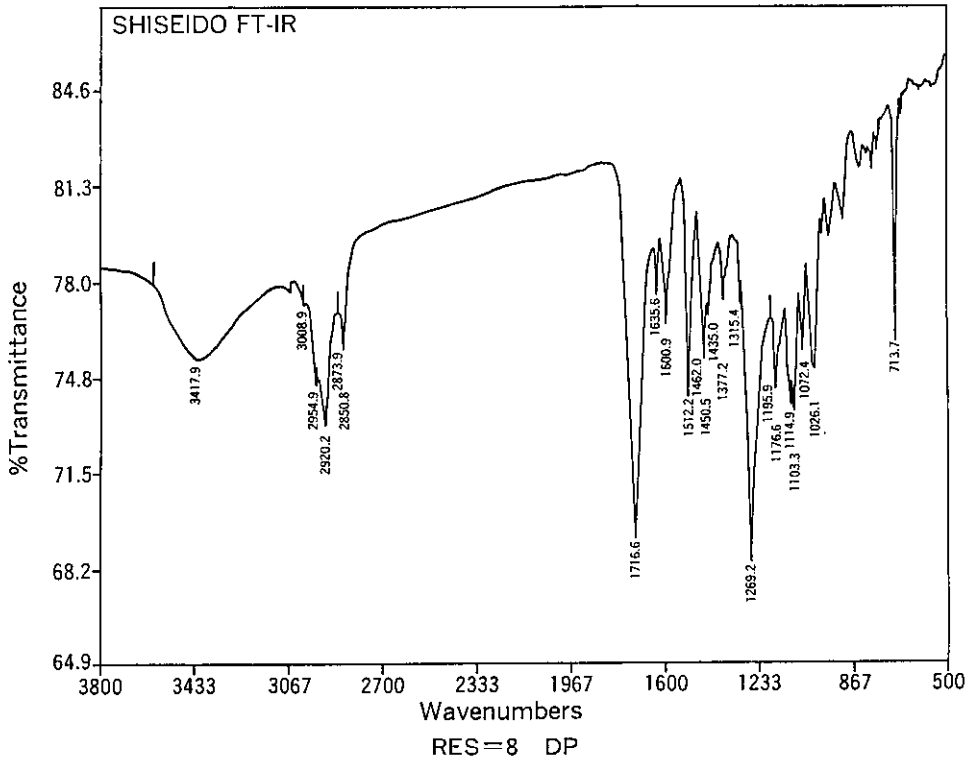


Figure 4. FT-IR spectrum of E

Table VIII. Allergenicity of Hypo-Allergenic Jasmin Oil in Guinea Pigs

Sample	Challenge test Concentration (%)	Results
Absolute jasmin oil	10	10/10, 3.2
Hypo-allergenic jasmin oil: Example 1	10	3/10, 0.3

Table IX. Contact Allergenicity of Jasmin Oils

Method: Human Maximization Test
Induction Concentration: 10%
Vehicle: Petrolatum

48 Hours After Challenge Application

Sample/Concentration(%)	10	5	3	1	0.5
Absolute Jasmin oil	6/23 (26.1%)	4/23 (17.4%)	2/23 (8.7%)	1/23 (4.3%)	1/23 (4.3%)
Hypo-allergenic Jasmin oil	1/15 (6.7%)	0/15	0/15	0/15	0/15

The biggest problem was whether the animal test results can be directly extrapolated to the risk in humans. The hypoallergenic jasmin oil tested in guinea pigs was evaluated in humans by Dr. Kligman and Mr. Kita of the University of Pennsylvania. The procedure of so-called human maximization test[5-8] is shown in figure 9.

Sodium lauryl sulfate in petrolatum was applied to either the back or upper arm of the subject. The application of SLS was performed three times during the procedure to enhance the penetration of the test samples. Ten percent of hypoallergenic jasmin oil in petrolatum was applied on the same site as the SLS treatment five times during three weeks as an induction procedure. Each induction consisted of a 48-hour occlusive patch test.

On the tenth day following the final induction

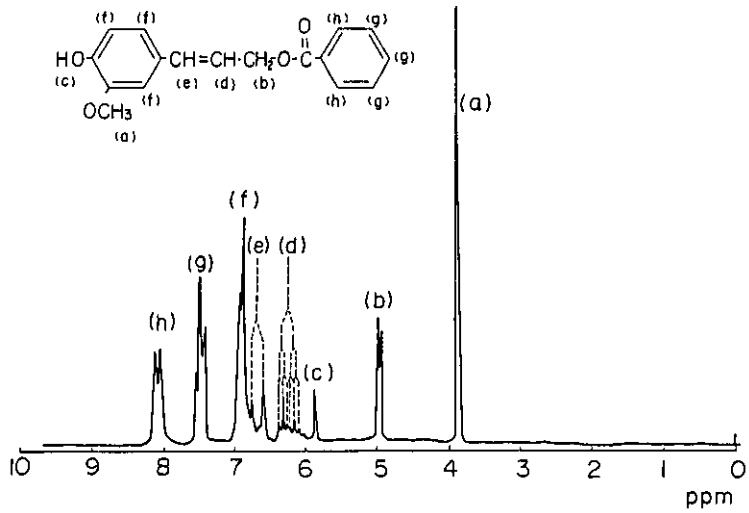


Figure 5. ^{1}H-NMR spectrum of E

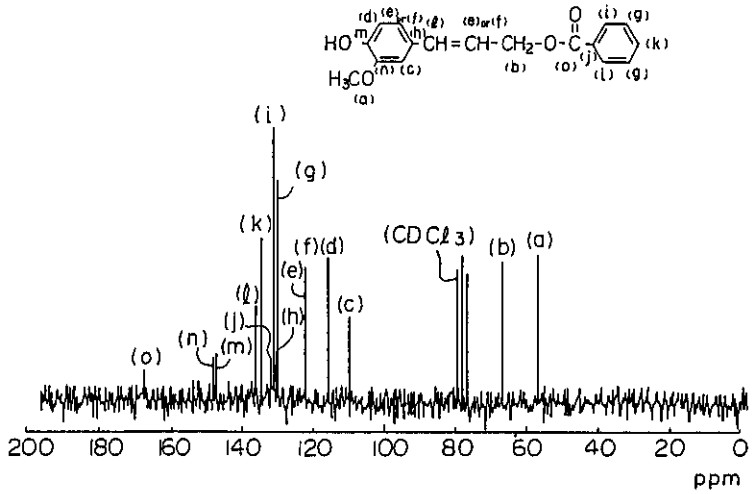
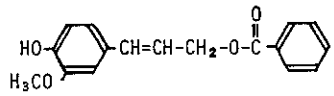


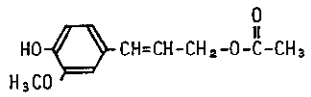
Figure 6. ^{13}C-NMR spectrum of E

procedure, challenge samples were applied to the forearm of the subjects. The reaction was evaluated at 48 and 72 hours following challenge application.

The results of absolute jasmin oil and hypoallergenic jasmin oil as evaluated by the human maximization test are shown in Table IX. The incidence of positive reaction to hypoallergenic jasmin oil was lower than that of absolute jasmin oil and the threshold concentration of hypoallergenic jasmin oil to elicit a positive reaction was higher than that of absolute jasmin oil. Thus we confirmed that the jasmin oil we developed is superb compared with natural jasmin oil with respect to its contact allergenicity.



(E)



(F)

Figure 7. Chemical formulae of coniferyl benzoate (E) and coniferyl acetate (F).

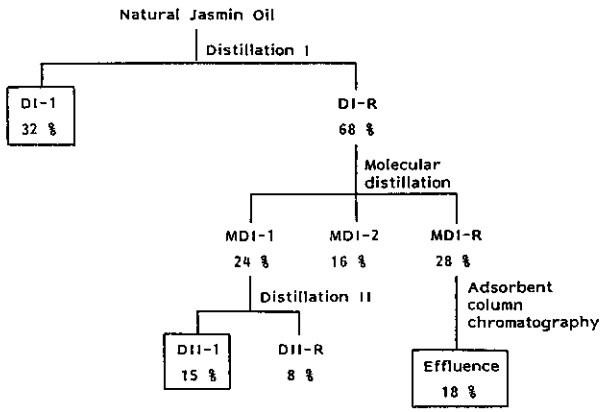


Figure 8. Procedure to obtain major components of hypoallergenic jasmin oil.

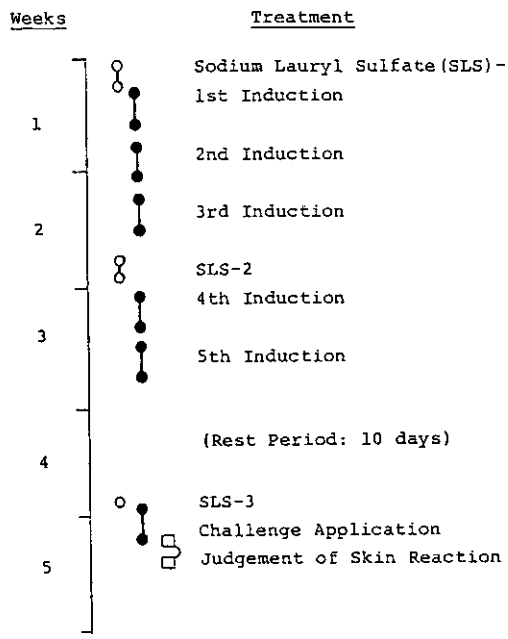


Figure 9. Maximization test procedure.

Conclusion

I have presented one of the examples of safe fragrance materials in cosmetic products developed and formulated by Shiseido. We must also turn our attention to problems of safety in fragrance materials other than allergenicity; we must consider dermal irritation and phototoxicity as well. Finally, I have to say that formulation of safe fragrance materials cannot be accomplished by cosmetic companies alone. The cooperation of perfumers, perfume companies and such organizations as RIFM (Research Institute for Fragrance Materials) and IFRA (International Fragrance Association) is sure to accomplish this goal.

Acknowledgement

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