Inhibition of IgE binding in ELISA was performed using a pool of sera from 24 HDM-sensitized patients having specific IgE to *D. pteronyssinus, D. farinae*, Der p 1 and Der p 2 above 3.5 kU_A/L, and IgE to cross-reactive carbohydrate determinants and bovine serum albumin below 1.0 kU_A/L, as measured by ImmunoCAP.

For each quantitative assay, Table S1 displays the number of dilution(s) applied to each batch of HDM liquid extract 300 IR/mL, the number of replicate(s) per dilution, the number or run(s) performed, as well as the intra-assay and inter-assay precisions.

Protein profiles were obtained using non-reducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using the Novex Midi Gel System (Thermo Fisher Scientific) according to manufacturer's instructions. All samples were diluted with NuPAGE LDS sample buffer (Thermo Fisher Scientific) so as to obtain 3 IR per lane. Electrophoresis was performed on NuPAGE 4-12 % gel with NuPAGE MES running buffer (Thermo Fisher Scientific). Proteins were then stained with SYPRO Ruby (Thermo Fisher Scientific) according to manufacturer's instructions. Protein profiles were obtained by acquisition of the fluorescent signal using a charge-coupled device (CCD) Camera Fusion FX7 (Vilber Lourmat).

Allergen profiles were obtained using western blotting after non-reducing SDS-PAGE. The latter was performed as described above, except that samples were diluted so as to obtain a 0.5 IR per lane in the case of group 2 allergen protein profiles. Proteins were then transferred by electroblotting onto a nitrocellulose membrane using the iBlot 2 Dry Blotting System (Thermo Fisher Scientific) according to manufacturer's instructions. The nitrocellulose membrane was incubated with KORI 75 murine mAb to both Der p 1 and Der f 1 (diluted at 1.5 µg/mL), with KORI 25 murine mAb to both Der p 2 and Der f 2 (diluted at 0.1 µg/mL), or with a pool of sera from HDM-sensitized individuals (diluted 1/50).

In the case of Der p 1 + Der f 1, the nitrocellulose membrane was incubated with Enhancer signal (Thermo Fisher Scientific). Detection of murine mAbs was performed using peroxidase-labelled sheep antibodies to mouse immunoglobulins (ref. A5906, Merck) diluted 1/5000. Detection of human IgE was performed using rabbit antibodies to human IgE (ref. A0094, Agilent), themselves detected using peroxidase-labelled goat antibodies to rabbit IgG (ref. 401393, Merck). After washings of the membrane, SuperSignalTM West Pico Plus substrate (ref. 34580, VWR) was added, and the allergen profile was obtained by acquisition of the chemiluminescent signal using a charge-coupled device (CCD) Camera (Vilber Lourmat).

For LC-MS/MS analyses, products were diluted at 10 IR/mL with 50 mM ammonium bicarbonate. All samples were prepared in 1 replicate. For each of them, 425 µL were placed in an Eppendorf tube and mixed with 3 µL of ProteaseMax 1 % (ref. V2072, Promega), and 1.5 µL of dithiothreitol 100 mM. Protein denaturation and reduction were performed by incubation at 56 °C for 20 min at 600 rpm in a Thermomixer (Eppendorf). Proteins were then alkylated with addition of 3 µL of iodoacetamide 100 mM and incubated at room temperature in the dark for 30 min. Excess of iodoacetamide was neutralized by addition of 1.5 µL of dithiothreitol 100 mM. Digestion was then performed by adding 13 µL of 50 mM ammonium bicarbonate and 2 µL of trypsin at 0.1 µg/µL. The solution was incubated at 37 °C for 3 h at 400 rpm in a Thermomixer. Digestion was stopped with addition of 2 µL of formic acid 100 % (final volume of 451 µL). The samples were then centrifuged at 25,000 *g* for 10 min. One hundred µL of supernatant were collected and deposited in LC-MS glass vials for LC-MS/MS analysis. Mass spectrometry was performed using an Ultimate 3000 RSLCnano liquid chromatography system (Thermo Scientific). Five 5 µL of each digest were loaded onto an Acclaim PepMap 100 C18 Trap column (Thermo Scientific) equilibrated at 50 °C with 1 % acetonitrile / 0.1 % trifluoroacetic acid and desalted for 2 min at a flow rate of 30 µL/min. Peptide separation was performed using a nanoEase M/Z HSS C18 T3 column (1.8 µm particle size, 100 Å, 75 µm x 10 cm,

Waters). The aqueous mobile phase (A) consisted of HPLC-grade water with 0.1 % formic acid, while the organic phase (B) was 80 % acetonitrile with 0.1 % formic acid. A gradient profile was used at a flow rate of 350 nL/min using the following linear gradient: 0-10 min, isocratic at 15% B; 10-42 min, from 15 to 40% B; 42-48 min, from 40 to 98% B; 48-54 min, isocratic; 54-54.1 min from 98 to 15% B; 54.1-63 min, isocratic. The column temperature was maintained at 50 °C. The column eluent was introduced into a Bruker QqToF Impact HD mass spectrometer equipped with a CaptiveSpray source (Bruker Daltonics). Analysis was performed in the positive ion mode. To ensure mass accuracy, the lock mass option was enabled in MS mode: m/z 1221.9906 ions (Chip cube high mass reference, Agilent Technologies) generated in the electrospray process from ambient air were used for internal recalibration. Internal recalibration was performed using DataAnalysis software (Bruker Daltonics, version 4.2). Identification of allergens was performed by matching acquired peptide tandem mass spectra to theoretical digests present in the *Dermatophagoides pteronyssinus* / *Dermatophagoides farinae* protein database downloaded from IUIS. Identification of non-mite proteins was performed with a second database downloaded from UniprotKB with proteins of nutrient media components (*Triticum aestivum* and *Saccharomyces cerevisiae*), usual nutrient media contaminants (*Aspergillus penicillioides* and *Penicillium rubens*), and human interference (keratin and trypsin).

Database search was performed with PEAKS software v8.5 (Bioinformatics Solutions Inc.) with the following parameters: peptide tolerance of 10 ppm (0.05 Da for fragment ions), trypsin enzyme, non-specific cleavage at one end of the peptide allowed, three missed cleavage permitted, carbamidomethylation of cysteine as a fixed modification, deamidation of asparagine, oxidation of methionine, pyro-glu on glutamic acid and HexNAcylation of asparagine as variable modifications, allowing a maximum of 4 variable post-translational modifications per peptide. Protein identifications were confirmed with the application of a 0.1 % peptide-spectrum match false discovery rate (estimated with the decoy fusion

method). When proteins were identified with four or less peptides, the spectrum patterns of these peptides were individually checked (signal, noise, fragmentation).

Table S1. Characteristics of the applied quantitative assays

Measured parameter	Number of tested dilution(s) per batch	Number of replicate(s) per dilution	Number of run(s)※	Intra-assay precision	Inter-assay precsision
TAA	1	2	1	5 %	7 %
Der p 1	2	2	1	2-3 %	5-9 %
Der f 1	2	2	1	2-3 %	3-6 %
Der p 2 + Der f 2	Osiris : 1 Staloral : 2	Osiris : 2 Staloral : 1	1	3-5 %	9 %
Der p 23	3	2	2	2-25 %	8 %
Protein	1	1	4	3 %	5 %

※: “1” means that all Staloral and Osiris batches were assayed in a same single run; “2” and “4” mean that the quantification of Staloral and Osiris batches was split into 2 and 4 runs, respectively

Table S2. Total allergenic activity, concentrations of Der p 1, Der f 1, group 2 allergens and Der p 23, and protein content in three batches of HDM Staloral 300 IR/mL and three batches of HDM Osiris 300 IR/mL, and resulting ratios

Product	Batch number	Total allergenic activity (IR/mL)	Der p 1 (µg/mL)	Der f 1 (µg/mL)	Der p 2 + Der f 2 (µg/mL)	Der p 23 (ng/mL)	Protein content (PNU※/mL)	Specific activity※※ (IR/PNU)
Staloral	2029254105	305	17.2	51.0	8.9	1,561	12,660	24.2
"DPTE/DFAR 50/50"	2029648941	333	16.3	47.1	8.1	1,720	12,300	27.1
300 IR/mL	2030007496	306	18.0	52.3	7.0	1,644	11,330	27.0
Mean		**315**	**17.2**	**50.1**	**8.0**	**1,642**	**12,097**	**26.1**
Osiris "Acariens mix 100 %" 300 IR/mL	0004409171	119	11.6	34.6	2.5	555	6,330	18.8
	0004414698	140	11.5	32.0	2.6	596	6,570	21.3
	0004451578	134	12.4	31.8	3.0	660	7,310	18.3
Mean		**131**	**11.8**	**32.8**	**2.7**	**604**	**6,737**	**19.5**
Ratio Staloral / Osiris		***2.4***	***1.5***	***1.5***	***3.0***	***2.7***	***1.8***	***1.3***

※: Protein nitrogen unit. ※※: Specific activity is defined as follows: [(total allergenic activity in IR/mL) / (protein content in PNU/mL)] × 1000