Supplementary Materials and Methods

Inhibition of IgE binding in ELISA was performed using a pool of sera from 11 cat-sensitized patients having specific IgE to cat dander and to Fel d 1 above 95 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 cat 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 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 2 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) caméra Fusion FX7 (Vilber Lourmat).

Allergen profiles were obtained using western blotting after reducing SDS-PAGE. The latter was performed as described above. 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 murine mAb of clone number 6F9 to Fel d 1 (diluted at 0.2 µg/mL), or with a pool of sera from cat-sensitized individuals (diluted 1/30). Detection of the mAb was performed using peroxidase-labelled sheep antibodies to mouse immunoglobulins (ref. A5906, Merck) diluted 1/5,000. Detection of human IgE was performed using rabbit antibodies to human IgE (ref. A0094, Agilent) dilted 1/2,000, themselves detected using peroxidase-labelled goat antibodies to rabbit IgG (ref. 401393, Merck) diluted 1/50,000. After washings of the membrane,

SuperSignalTM West Pico Plus substrate (ref. 34580, Thermo Fisher Scientific) was added, and the allergen profile was obtained by acquisition of the chemiluminescent signal using a CCD camera Fusion FX7 (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 a NanoElute2 liquid chromatography system (Bruker). Five 5 µL of each digest were loaded onto an Acclaim PepMap 100 C18 Trap column (Thermo Scientific) and desalted for 10 column volumes at a pressure of 70 bar. Peptide separation was performed using a PepSep Series C18 column (1.9 µm particle size, 100 Å, 75 µm x 10 cm, Bruker). 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: 0-30 min, from 2 to 40% B; 30-31 min, from 40 to 95% B; 31-35 min, isocratic at 95% B. The column temperature was maintained at 40 °C. The column eluent was introduced into a Bruker TimsTOF Pro2 mass spectrometer equipped with a CaptiveSpray2 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 generated

in the electrospray process from ambient air were used for internal recalibration. Identification of allergens was performed by matching acquired peptide tandem mass spectra to theoretical digests present in the *Felis domesticus (F. catus)* protein database downloaded from IUIS (updated in September 2023) and UniprotKB database for *Felis catus* (updated in October 2023).

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).

Statistical comparison of cat Staloral and Osiris 300 IR/mL products was performed using unpaired t test with Welch's correction, after checking the normal distribution of the data of each group to be compared using the Shapiro-Wilk normality test. All statistical tests were performed using Prism software version 10 (GraphPad Software, La Jolla, California, USA). A difference between two groups was considered significant when the *p*-value was strictly below 0.05.

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 precision
TAA	1	2	1	12 %	12 %
Fel d 1 content***	5	2	1	3 %	9 %
Fel d 4 content***	4	2	1	4-8 %	6 %
Protein content	1**** or 3*****	1	3	3 %	5 %

* Those characteristics were obtained internally for TAA and the contents in Fel d 1 and protein. With respect to Fel d 4 content, the characteristics were obtained from InBio (see https://inbio.com/wp-content/uploads/2022/12/ELISA-2.0-Performance-Characteristics-26-Allergens-121322-update-Inhalable.pdf).

** "1" means that all Staloral and Osiris batches were assayed in a same single run; "3" means that the quantification of Staloral and Osiris batches was split into 3 runs.

*** Quantified using commercially available ELISA (Inbio, Charlottesville, VA, USA).

**** For cat Staloral 300 IR/mL batches.

***** For cat Osiris 300 IR/mL batches.

Table S2. Total allergenic activity, concentrations of Fel d 1 and Fel d 4, and protein content in three batches of cat Staloral 300 IR/mL and three batches of cat Osiris 300 IR/mL, and resulting ratios

Product	Batch number	Total allergenic activity (IR/mL)	Fel d 1 (µg/mL)	Fel d 4 (ng/mL)	Protein content (PNU*/mL)	Specific activity**
Staloral "chat" 300 IR/mL	2032341132	318	105.9	438.4	14,550	21.9
	2032341139	365	105.6	442.7	15,810	23.1
	2032341141	356	108.2	448.1	15,900	22.4
Mean		**346**	**106.6**	**443.1**	**15,420**	**22.4**
Osiris "Chat (phanères)" 300 IR/mL	0004551863	139	93.7	271.2	9,900	14.0
	0004560942	189	76.6	217.9	9,880	19.1
	0004562818	192	77.8	218.3	9,850	19.5
Mean		**173**	**82.7**	**235.8**	**9,877**	**17.6**
Ratio Staloral / Osiris		***2.0***	***1.3***	***1.9***	***1.6***	***1.3***
Statistical difference between cat Staloral and Osiris 300 IR/mL		**Yes ($p < 0.01$***)**	**Yes ($p < 0.05$***)**	**ND******	**Yes ($p < 0.01$***)**	**No ($p > 0.05$***)**

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

*******: Two-tailed *p*-value. ********: Not determined, because the data of tested Osiris batches did not pass the normality test.