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Sevoflurane: Impurities and stability testing

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ABSTRACT

We report an impurities and stability testing study on two different batches of three different commercial formulations of sevoflurane, the widely used fluorinated inhalation anesthetic agent. Our analyses focused on identifying the starting presence of volatile impurities as well as the formation of degradants after storage also under accelerated ageing conditions. We found that the analyzed samples show differences in quality and quantity of impurities as a likely consequence of the different manufacturing processes. Impurities may vary from one batch to the other of a given formulation, but in all case they are well below limits specified by regulatory agencies for clinical use, the content in sevoflurane being always > 99.99%. Fluoride anion concentrations were below 0.1 mg/L in all analyzed samples, consistent with no degradation occurring in the examined timeframe.

1. Introduction

Several fluorinated ethers, such as desflurane, enflurane, isoflurane, and sevoflurane, are used as inhalation anesthetic agents in modern clinical practice [1]. Among these, sevoflurane, *i.e.*, 1,1,1,3,3,3-hexafluoro-2-(fluoromethoxy)propane (Scheme 1), is the most commonly used because of its rapid onset of action and the quick recovery from anesthesia induced by this agent [2]. Sevoflurane is produced by several manufacturers. Currently marketed sevoflurane formulations (**SFs**) differ in their methods of synthesis [3], and the anesthetic is frequently prepared from hexafluoroisopropanol (**HFIP**) *via* a one-step synthesis (by using formaldehyde and HF under acid catalysis, Scheme 2), or *via* a three-step synthesis, sevomethyl ether $(CF_3)_2CH\text{-}O\text{-}CH_3$ (**SME**) and chlorosevo ether $(CF_3)_2CH\text{-}O\text{-}CH_2Cl$ (**CSE**) being reaction intermediates. A two-step synthesis (chloromethylation of **HFIP** then fluorine for chlorine substitution) has also been developed. While the actual processes for producing marketed sevoflurane are trade secrets, patent literature and published articles may enable for surmising the employed chemistry. Likely reagents are: a) three-step synthesis: Dimethylsulfate as the methylating agent, chlorine gas with photochemical activation as the chlorinating agent, and a bulky tertiary amine·HF salt as the fluorinating agent; b) Two-step synthesis: Aluminum trichloride and 1,3,5-trioxane as chloromethylating agent; c) One-step synthesis: Sulfuric acid and fluorosulfuric acid as acid catalysts and dehydrating agents. Marketed **SFs** differ also in containers in which they are sold (Fig. S1) and water content [4].

For an active pharmaceutical ingredient (API), impurities generated during the manufacturing process have to be decreased to levels ultimately specified by the due regulations, *e.g.*, the European Pharmacopoeia (EP). EP 9.0 classifies three compounds, encoded as **A**, **B**, and **C**, as sevoflurane impurities [5] (Scheme 1). Fluoromethyl 2,2-difluoro-1-(trifluoromethyl)vinyl ether (compound **A**) may derive from degradation of sevoflurane by dehydrofluorination [6]. It is regarded as an impurity that should not exceed 25 ppm limit. **SME** (*i.e.*, compound **B**) is a reaction intermediate in the three-step industrial synthesis of sevoflurane [7] and should not exceed 100 ppm limit according to the EP 9.0. Both compounds **A** and **B** are classified by the EP 9.0 as "*specified impurities*". **HFIP** (*i.e.*, compound **C**) is the starting material of the three industrial synthetic processes of sevoflurane mentioned above, and one of the possible degradation products of sevoflurane *via* Lewis acid catalysis [7]. Compound **C** is classified by the EP 9.0 among "*other detectable impurities*", *i.e.*, potential impurities with a defined structure but not known to be normally present above the identification threshold in substances used in medicinal products that have been authorized by the competent authorities. The maximum limit for impurity **C** is 100 ppm. Other "*unspecified impurities*" may be present, but it's not necessary to identify them for demonstration of compliance. However, for each of them the maximum limit is 100 ppm, and the

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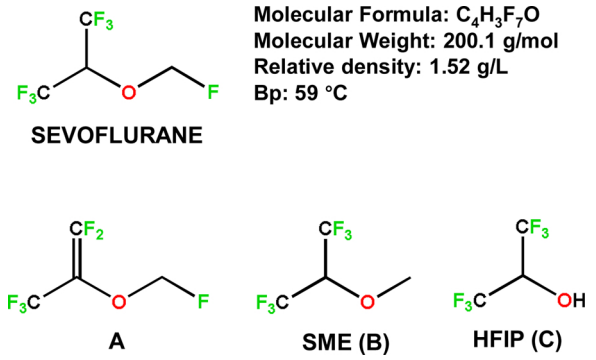
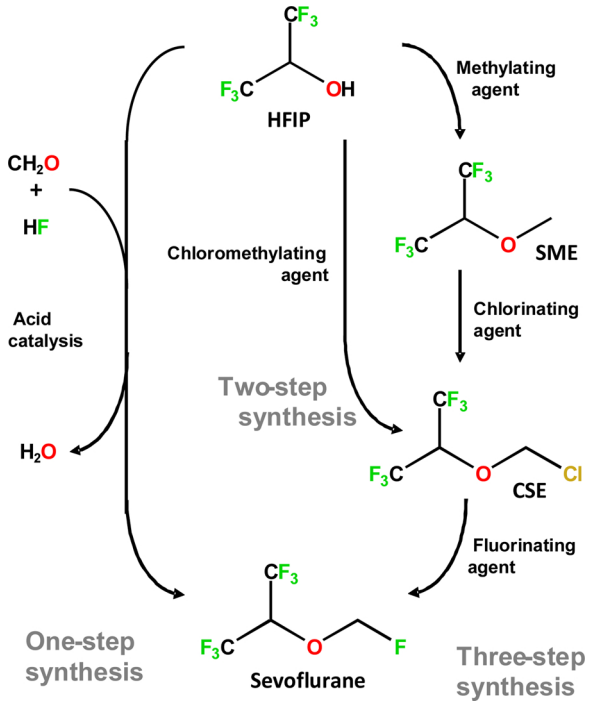


Scheme 1. Top: Chemical structure of sevoflurane and its main physico-chemical properties. Bottom: Chemical structures of sevoflurane impurities encoded as **A**, **B**, and **C**, according to the EP 9.0.



Scheme 2. Typical manufacturing processes of sevoflurane. According to EP 9.0, sevomethyl ether (**SME**) and hexafluoroisopropanol (**HFIP**), are encoded as impurities **B** and **C**, respectively.

maximum limit for total impurities in sevoflurane for medical applications is 300 ppm [5].

While most of the literature in this field focuses on the study of sevoflurane degradation profile when stored in anesthesia vaporizers [8], to the best of our knowledge, reports on comparative analysis of the stability of sevoflurane formulations produced by different manufacturers are less than a handful [9]. In previous works, manufacturing impurities were found not to be quantitatively and clinically significant, as long as they remain low [7]. The objective of our study has been to analyze three different marketed formulations of sevoflurane and to determine the level of volatile impurity after conservation at different storage conditions. The three analyzed **SFs** differ by the water content declared by the respective manufacturers, **SF1** and **SF2** being the lower-water sevoflurane formulations (≤ 130 ppm), **SF3** being the higher-water sevoflurane formulation (> 300 ppm) (see ESI) [2,4,7]. This is relevant because sevoflurane is known to decompose in the presence of Lewis acids, and water functions as a Lewis acid inhibitor [10].

Our analyses first focused on identifying the possible presence of EP 9.0 impurities **A-C** (Scheme 2), and/or other manufacturing impurities and/or formed degradants, then quantifying them, and trying to analyze the causes of their eventual presence. For the latter objective, we measured the concentration of fluoride (F^-) ions in the samples, which is a hallmark of sevoflurane degradation [8b]. The analyses were carried out on samples from two different production batches, **batch-1** and **batch-2**, stored under normal temperature, relative humidity (RH), and lighting conditions (***std***), and under accelerated ageing conditions (***aa***). The present study was conducted independently of the companies manufacturing and selling the products.

2. Results and discussion

2.1. GC–MS analyses: Volatile organic impurity determination and stability studies

For the separation, identification, and quantification of manufacturing impurities and degradation products, we relied on Gas Chromatography-Mass Spectrometry (GC–MS) analysis. Our analytical method was validated against commercial samples of compounds **A**, **B**, and **C**, which were used for the precise calibration of the instrument response and the qualitative and quantitative identification of these compounds in sevoflurane formulations possibly containing them in unknown concentrations. Five replicas of analyses were performed for any point in the calibration curve (Figs. S3-5 in Supplementary Material). The three sevoflurane related compounds were clearly separated under the used conditions (Fig. 1), and did not overlap with the peak of pure sevoflurane, which had, when present in large quantities, a retention time of 6.0–6.9 min. (Fig. 2, missing baseline region).

The three analyzed sevoflurane formulations **SF1**, **SF2**, and **SF3** are marketed in bottles made of different materials (epoxy-phenolic resin lined aluminum to prevent sevoflurane degradation [7] by the oxide formed at the surface, glass, and polyethylene napthalate, PEN, respectively, Fig. S1) and have different declared shelf-lives, *i.e.*, 2 years, 5 years, and 3 years, in the order. In all cases, no special storage instructions are advised, but to store below 30 °C and to keep the cap tightly closed. For this reason and in order to mimic hospital storage conditions, we first analysed the three formulations after storing them at ambient conditions in a fume hood with an average temperature of

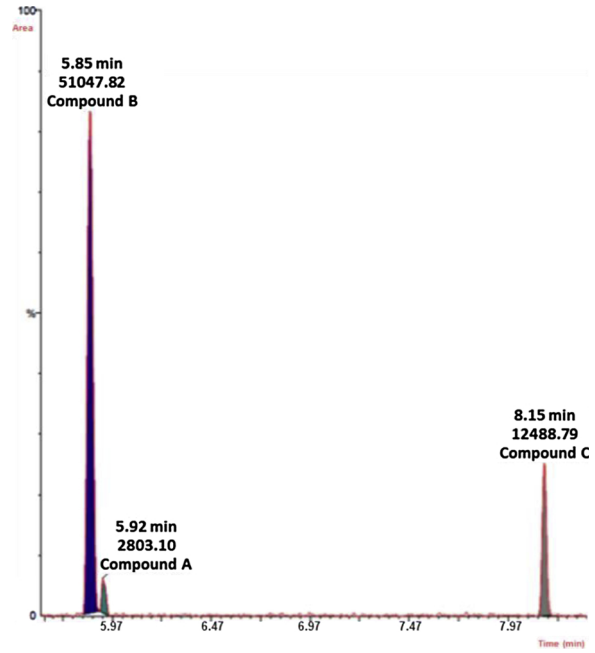


Fig. 1. GC–MS chromatogram of a mixture of sevoflurane "*specified impurities*" **A** (28 mg/kg), **B** (69 mg/kg), and **C** (76 mg/kg) prepared from commercial samples.

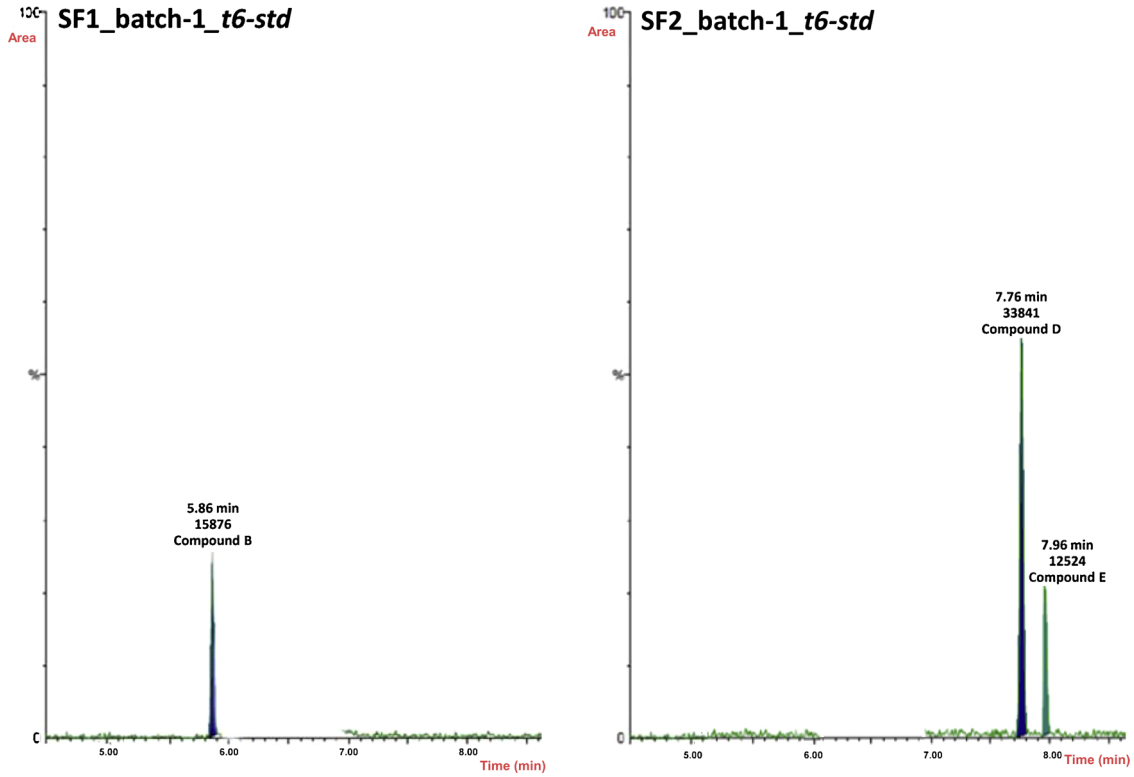


Fig. 2. GC–MS chromatograms of **SF1_batch-1_*t6-std*** sample (left) and **SF2_batch-1_*t6-std*** sample (right). The baseline is missing in the sevoflurane time window (6.0–6.9 min region) as the filament was turned off to protect it during the out-of-scale signal.

24 °C and average RH of 36% (standard conditions, ***std***) for 6 months (***t6***), namely within the expected product shelf-lives. All formulations were injected as pure liquids in order to maximize the sensitivity of the method and possibly detect very low impurity concentrations.

Interestingly, **SF1_batch-1_*t6-std*** contained impurity **B**, *i.e.*, **SME**, at a concentration of 12.2 ± 5 ppm (Fig. 2, left), which is well below the limit accepted by the EP 9.0, *i.e.*, 100 ppm (Table 1). **SME** is an intermediate of a three-steps sevoflurane synthesis [7], therefore **B** found in **SF1_batch-1_*t6-std*** may derive from the manufacturing process. **SF2_batch-1_*t6-std***, instead, was found to contain two impurities that were different from **A**, **B**, and **C** (Fig. 2, right) and we classified them as **D** and **E**. These two impurities have retention times of 7.76 and 7.96 min, respectively, namely they appear after **A**, **B**, and sevoflurane (retention times of which are 5.85, 5.92, and 6.0–6.9 min. in the order) and before **C** (having a retention time of 8.15 min.). Moreover, the **D**/**E** ratio of peak area integrations was 2.7. Finally, in the adopted analytical conditions, no detectable organic volatile impurities was found in **SF3_batch-1_*t6-std*** (Fig. S6, left). This is remarkable, and may be associated to either the adopted synthetic, or purification processes for manufacturing **SF3**, or both procedures.

In order to mimic longer-term room temperature storage conditions, new samples of the same batch as above were stored under accelerated ageing (***aa***) conditions, *i.e.*, 40 °C, 75% RH, for 6 months (***t6***) and then analyzed by GC. **SF1_batch-1_*t6-aa*** was found to contain impurity **B** at a concentration of 8.5 ± 5 ppm (Fig. S7, left). This amount is very similar to that of **SF1_batch-1_*t6-std*** sample, demonstrating that the quantity **B** present in the two samples is largely unaffected by the storage conditions, and remain significantly lower than limits specified by EP 9.0, even after storage under conditions more severe than those recommended by the manufacturer. The quantity of **B** does not increase over time and this suggests that B derives from the industrial synthesis of sevoflurane rather than from its decomposition. Analogously, **SF2_batch-1_*t6-aa*** contained the unknown impurities **D** and **E** found in the **SF2_batch-1_*t6-std*** sample with retention times of 7.76 and

Table 1
Amounts of volatile impurities detected *via* GC–MS in different batches of sevoflurane formulations **SF1**, **SF2**, and **SF3** stored for different times under different conditions.

Sample	Impurities[a]			
	B	D	E	F
SF1_batch-1_*t6-std*	12.2 ± 5[b]	–	–	–
SF2_batch-1_*t6-std*	–	21.1[c]	9.6[c]	–
SF3_batch-1_*t6-std*	–	–	–	–
SF1_batch-1_*t6-aa*	8.5 ± 5[b]	–	–	–
SF2_batch-1_*t6-aa*	–	17.5[c]	7.3[c]	–
SF3_batch-1_*t6-aa*	–	–	–	–
SF1_batch-1_*t9-std*	9.3 ± 2[b]	–	–	–
SF2_batch-1_*t9-std*	–	8.4[c]	2.8[c]	–
SF1_batch-1_*t9-aa*	6.5 ± 2[b]	–	–	–
SF2_batch-1_*t9-aa*	–	9.6[c]	3.2[c]	–
SF1_batch-2_*t0*	8.6 ± 2[b]	–	–	–
SF2_batch-2_*t0*	26.1 ± 0.2[b]	30.6[c]	– –	11.4[c]
SF3_batch-2_*t0*	–	–	–	–
SF1_batch-2_*t3-std*	22.6 ± 2[b]	–	–	–
SF2_batch-2_*t3-std*	31.0 ± 0.2[b]	28.5[c]	–	12.6[c]
SF3_batch-2_*t3-std*	–	–	–	–
SF1_batch-2_*t3-aa*	17.3 ± 2[b]	–	–	–
SF2_batch-2_*t3*-aa	30.3 ± 0.2[b]	27.7[c]	–	13.2[c]
SF3_batch-2_*t3*-aa	–	–	–	–

[a] Retention times (min.) are: Sevoflurane, 6.0–6.9; B, 5.92; D, 7.76; E, 7.96; F, 7.00.

[b] Amount in ppm, quantified by peak integration and calibrated with respect to an authentic pure sample. Uncertainty values for B presented in Table 1 are estimated based on standard deviations of multiple analyses.

[c] Amount in ppm, quantified by peak integration and calibrated with respect to an authentic pure sample of **B** (used as internal standard for batch-2 samples and as external standard for batch-1 samples) and assuming a similar detector response. Standard deviation is not given in relation to approximations inherent in calibration protocol.

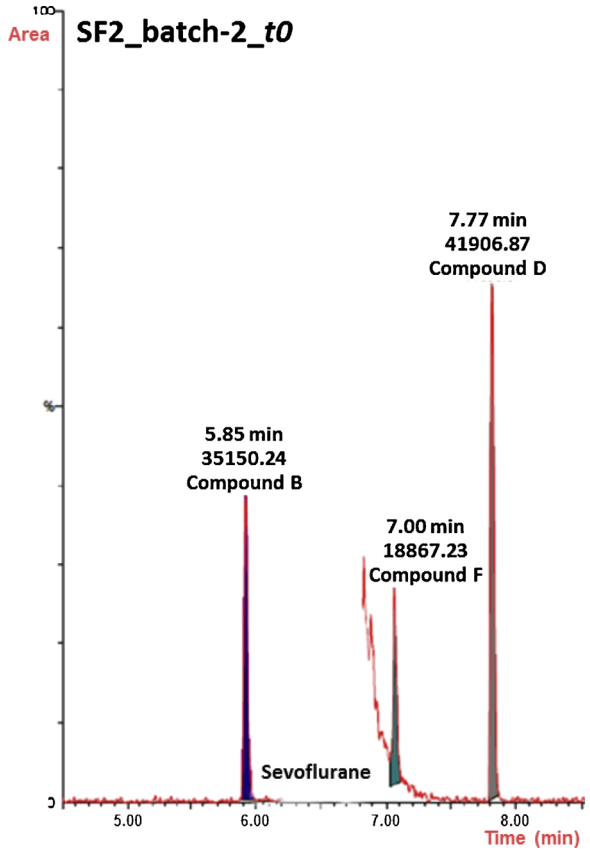


Fig. 3. GC–MS chromatogram of **SF2_batch-2_*t0***. The baseline is missing in the 6.0–6.8 min region (sevoflurane time window) as the filament was turned off to protect it during the out-of-scale signal.

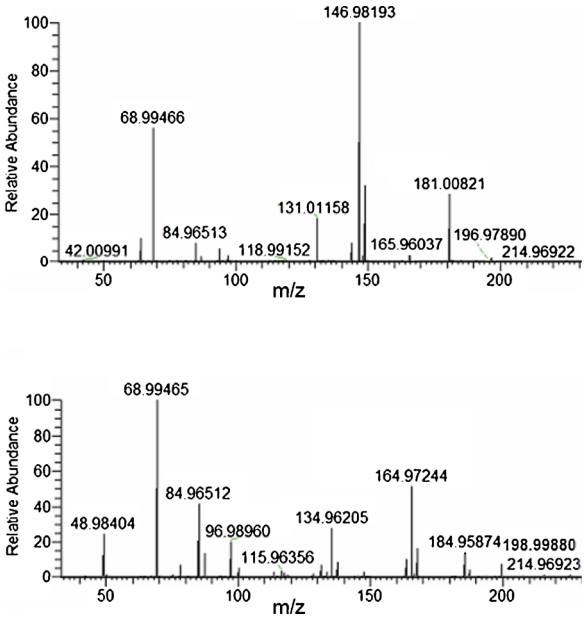


Fig. 4. HRMS spectra (electron impact, 70 ev ionizing energy) of **D** (top) and **E** (bottom) impurities.

7.96 min at similar absolute concentrations and with a similar **D**/**E** ratio of peak area integrations, *i.e.*, 2.4 (Fig. S7, right). Clearly, the absolute and relative concentration of impurities is independent of the storage conditions also in **SF2_batch-1**. Finally, **SF3_batch-1_*t6-aa*** (Fig. S6, right) did not show the presence of any organic volatile impurities detectable in the adopted analytical conditions, again similar to the **SF3_batch-1_*t6-std*** sample. This confirms that **SF3_batch-1** does not contain impurities coming from the industrial synthetic process, and that volatile organic degradation products do not form in its samples under the adopted storage conditions.

Finally, the batches showing the presence of impurities, namely **SF1_batch-1** and **SF2_batch-1**, were also analyzed after ageing 9 months (***t9***) under ***std*** and ***aa*** storage conditions. **SF1_batch-1_*t9-std*** and **SF1_batch-1_*t9-aa*** showed **B** concentrations of 9.3 ± 2 ppm and 6.5 ± 2 ppm, respectively and these values, within the experimental error, are perfectly in line with concentrations of ***t6*** samples (Table 1 and Fig. S8, top). Analogously, both **SF2_batch-1_*t9*** samples showed the same impurities found in corresponding ***t6*** samples, *i.e.*, impurities **D** and **E**, in a 3:1 **D**/**E** ratio and at an overall reduced absolute concentration (Table 1 and Fig. S8, bottom). This confirms that impurities found in **SF1_batch-1** and **SF2_batch-1** do not show any dynamics dependent on samples storage duration and conditions.

The fact that impurities found in the samples are independent of the storage conditions, supports the hypothesis that they are production instead of degradation impurities, and we thus decided to analyze samples coming from another production batch (**batch-2**). We performed GC experiments on samples as soon as delivered from the manufacturers (***t0***) and after 3 months (***t3***) of storage under ***std*** and ***aa*** conditions. Samples of **SF1_batch-2**, again showed the presence of impurity **B**, at concentrations of 8.6 ± 2 ppm (***t0***), 22.6 ± 2 ppm (***t3-std***), and 17.3 ± 2 ppm (***t3-aa***) (Table 1 and Fig. S9). Despite a small increase of the **B** concentrations in the stored samples, these are still well below the limit set by the EP 9.0, *i.e.*, 100 ppm. Surprisingly, the set of peaks found in **SF2_batch-2** was different from that found in **SF2_batch-1**. In fact, all three analyzed samples (***t0***, ***t3-std***, and ***t3-aa***) showed three peaks at 5.85 min., 7.00 min., and 7.79 min. retention times (Table 1, Fig. 3, and Fig. S10). The peak eluting at 5.85 min. is ascribed to the impurity **B**, and is now present at concentrations of 26.1 ± 0.2 ppm (***t0***), 31.0 ± 0.2 ppm (***t3-std***), and 30.3 ± 0.2 ppm (***t3-aa***). The peak at 7.79 min. is ascribed to impurity **D**, and, in **SF2_batch-2_*t0*** sample, is present at a concentration level close to those of **SF2_batch-1_*t6*** samples. The peak at 7.00 min., instead, is a new impurity which was not found in samples of the previous batch and is here encoded as **F**. The relative ratio **B**/**F**/**D** of peak area integrations was roughly 2:1:2. Clearly, **SF2** shows a certain variability in impurities composition from batch to batch, with **D** being the major and only impurity in common between the two batches. By considering impurity **B** as an internal standard, impurities **D** and **F** were quantified by peak integration assuming a similar detector response to that of **B**.

The concentrations of **D** and **F** in **SF2_batch-2** samples, averaged over the three analyses performed at different times/storage (Table 1), were of 27 and 12 ppm, respectively, once again well below EP 9.0 limits (Fig. S10). Finally, none of the **SF3_batch-2** samples showed any detectable organic volatile impurities in the adopted analytical conditions (Fig. S11), again similarly to all previous samples.

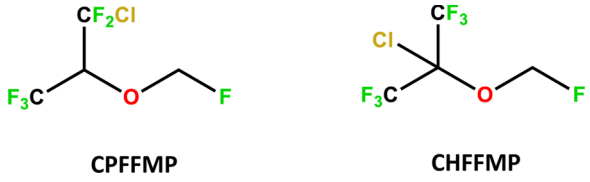
In order to possibly determine the structures of impurities **D-F**, we resorted to high-resolution GC-HRMS (Mass Spectrometry) analysis with the Thermo Scientific^{TM} Q Exactive^{TM} GC Orbitrap^{TM} GC–MS/MS system to obtain their exact masses (Fig. 4 and Fig.s S14-15). The GC-Orbitrap-based high resolution accurate mass instrumentation offers the ability to obtain mass spectral data at high resolving power with mass accuracies < 1 ppm.

Table 2 gives the major peaks that were considered for the assignments. There is a potential for rearrangements in mass spectrometry and identified ions may not match the actual compounds under study, but considering the molecular ion peak at *m/z* 216.9848 in chemical ionization (CI) and at 214.9692 in electron impact (EI), the major impurity seen in both batches of **SF2** samples, *i.e.*, **D** at 7.76 min., may be ascribed to 1-chloro-1,1,3,3,3-pentafluoro-2-(fluoromethoxy)propane, **CPFFMP** (Scheme 3). This assignment is supported by the peak at *m/z* 196.9789 $[MW\text{-}F]^+$ and 181.0082 $[MW\text{-}Cl]^+$ and the presence of

Table 2
Exact masses and corresponding formulas of main peaks in HRMS spectra of **D** and **E** impurities. If not otherwise specified, all peaks were observed in EI mode.

	Exact Mass	Molecular formula	Peak assignment
Peak D (7.76 min.), CPFFMP (MW: 216) $C_4H_3ClF_6O$	216.9848[a,b]	$C_4H_4ClF_6O$	$[MW + 1]^+$
	214.9692[a]	$C_4H_2ClF_6O$	$[MW-1]^+$
	196.9789[a]	$C_4H_3ClF_5O$	$[MW-F]^+$
	181.0082	$C_4H_3F_6O$	$[MW-Cl]^+$
	146.9819[a]	$C_3H_3ClF_3O$	$[MW-CF_3]^+$
	131.0116	$C_3H_3F_4O$	$[MW-CF_2Cl]^+$
	96.9896	C_2F_3O	$[CF_3C=O]^+$
	84.9651[a]	$CClF_2$	$[CF_2Cl]^+$
	68.9947	CF_3	$[CF_3]^+$
Peak E (7.98 min.), CHFFMP (MW: 234) $C_4H_2ClF_7O$	214.9692[a,b,c]	$C_4H_2ClF_6O$	$[MW-F]^+$
	198.9988	$C_4H_2F_7O$	$[MW-Cl]^+$
	184.9587[a]	C_3ClF_6	$[MW-CH_2FO]^+$
	164.9724[a]	$C_3H_2ClF_4O$	$[MW-CF_3]^+$
	96.9896	C_2F_3O	$[CF_3C=O]^+$
	84.9651[a]	$CClF_2$	$[CF_2Cl]^+$
	68.9946	CF_3	$[CF_3]^+$
	48.9840	CH_2FO	$[FCH_2-O]^+$

[a] Corresponding peak with the less abundant ^{37}Cl isotope was also observed.
[b] Peak observed in chemical ionization (CI).
[c] Peak observed both in electron impact (EI) and in chemical ionization (CI).



CPFFMP CHFFMP

Scheme 3. Proposed chemical structures of impurities **D** (CPFFMP) and **E** (CHFFMP).

complementary ions (*i.e.*, $[MW-CF_3]^+$ and $[CF_3]^+$, $[MW-CF_2Cl]^+$ and $[CF_2Cl]^+$). **CPFFMP** may form *via* dehydrofluorination of sevoflurane to fluoromethyl 2,2-difluoro-1-(trifluoromethyl)vinyl ether (compound encoded as impurity **A** according to EP 9.0) and addition of hydrogen chloride. Alternatively, **CPFFMP** may come from free chloride in the HalEx reactions or during the chlorination of **SME** in a multistep sevoflurane synthesis [11,13]. For sake of information completeness, it may be interesting to note that **CPFFMP** has already been described as the mayor byproduct in a patented sevoflurane synthesis where the agent is obtained from methyl, or chloromethyl, 1,1,1,3,3,3-hexachloropropyl ether *via* fluorine for chlorine substitution [12]. **CPFFMP** is a structural isomer of chlorosevo ether (**CSE**, Scheme 2), which is an intermediate in the three-steps synthesis of sevoflurane. However the correspondence between **D** and **CSE** could certainly be excluded as the retention time of the latter compound, in the same experimental conditions, is 8.83 min. (Fig. S12), and the mass fragmentation patterns of the two derivatives are different (Fig. S13).

The mass spectrometric and high resolution analysis has also revealed that the impurity **E** seen in the **SF2_batch-1** samples at 7.97 min., has a fragmentation pattern compatible with 2-chloro-1,1,1,3,3,3-hexafluoro-2-(fluoromethoxy)propane, **CHFFMP** (Scheme 3, Table 2). While other regioisomers might be compatible with the observed fragmentation pattern, the presence of complementary ions (*i.e.*, $[MW-CF_3]^+$ and $[CF_3]^+$, and $[MW-CH_2FO]^+$ and $[CH_2FO]^+$) supports the proposed assignment, along with the $[MW-F]^+$ and $[MW-Cl]^+$ peaks.

Other minor process impurities deriving from partial halogenation, hydrolysis, addition, and fragmentation reactions may be present in very small quantities, such as the impurity **F**, whose low-resolution MS fragmentation peaks indicate a structure compatible with a polyfluorinated derivative, possibly generated during the synthesis or from hydrolysis and addition reactions, as well (Fig. S16). The structural similarity between **B**, **D**, and **E** impurities validates the assumptions used for quantification of these compounds (Table 1, notes b and c).

2.2. Ion–Exchange Chromatography analysis of anions

Hydrogen fluoride (HF) is both a reactant of the one-step sevoflurane industrial synthesis and a product formed upon Lewis acid-catalyzed degradation of this anesthetic. Moreover, HF may promote further formation of Lewis acids by etching aluminum or glass containers. For this reason, HF is considered a hallmark of sevoflurane degradation, and F^- concentration is therefore routinely measured for its assessment [8b]. We measured F^- concentration against a standard solution, containing known concentrations of various anions, after sevoflurane extraction with water and by using Ion-Exchange chromatography (IC) as the analytical method (Figs. S17-19 in Supplementary Material).

All sevoflurane samples from the different suppliers, different production batches, and different storage conditions discussed above and listed in Table 1 were analyzed to assess possible F^- presence and its concentrations and none of them revealed a presence higher than 0.1 mg/L. This value is perfectly within the limit that the EP 9.0 sets for F^- concentration (2 mg/L), and supports conclusions drawn from volatile products study that degradation did not occur in the studied samples during the observation period, irrespective of the storage conditions. Moreover, this result is well in line with the hypothesis formulated above that found impurities **B** and **D-F** are more likely to be coming from the synthetic process rather than from degradation reactions.

Interestingly, our method was also able to spot the presence of small quantities of other anions in all sevoflurane samples, such as chloride (Cl^-) and nitrate (NO_3^-) ions, and their concentrations are much greater than those of F^- (Table 3). These other anions may derive from environmental contamination.

2.3. Karl–Fischer analysis

Finally, we measured starting water content in sevoflurane samples before ageing by using the Karl-Fischer method. Water, in sufficient quantity, may help in inhibiting Lewis acid-dependent sevoflurane degradation [7,10]. Water can be present in sevoflurane samples due to the adopted industrial process, but the protective effect of water mentioned above prompted a producer to add water in his final sevoflurane formulation to produce a so-called “water-enhanced” or “wet” sevoflurane. Formulations **SF1-SF3** differ for their water contents declared by the manufacturers, **SF1** and **SF2** being the lower-water sevoflurane formulations ($\leq$ 130 ppm), **SF3** being the higher-water sevoflurane formulation ($>$ 300 ppm) (see ESI) [2,4,7]. Importantly, we found a good correspondence between these literature data and measured values on ***t0*** and ***t6*** samples (Table 4). At least 5 replicas of analyses were performed for each sample in order to provide a more accurate standard deviation on the data.

Table 3
Concentrations of fluoride, chloride, and nitrate anions determined by ion-exchange chromatography in some representative batches. Similar concentrations were obtained for other batches.

Batch	F^- (mg/L)	Cl^- (mg/L)	NO_3^- (mg/L)
SF1_batch-1_*t6-std*	< 0.1	0.745	0.715
SF2_batch-1_*t6-std*	< 0.1	1.197	1.091
SF3_batch-1_*t6-std*	< 0.1	0.586	0.512
SF1_batch-1_*t6-aa*	< 0.1	0.243	0.304
SF2_batch-1_*t6-aa*	< 0.1	0.382	0.478
SF3_batch-1_*t6-aa*	< 0.1	0.374	0.428

Table 4
Water content (Karl-Fisher) in some of studied batches.

SF sample	Water content[a] (ppm)
SF1_batch-1_*t6-std*	50.8 ± 20.0
SF2_batch-1_*t6-std*	65.2 ± 20.0
SF3_batch-1_*t6-std*	527.6 ± 50.0
SF1_batch-2_*t0*	25.9 ± 5.0
SF2_batch-2_*t0*	27.2 ± 5.0
SF3_batch-2_*t0*	498.8 ± 50.0

[a] Uncertainty values presented in Table 4 are estimated based on standard deviations of multiple analyses.

3. Conclusions

Two production batches of sevoflurane samples from three different manufacturers were stored under standard and accelerated ageing conditions and analyzed at different ageing times. The analyzed samples show differences in quality and quantity of impurities, which are present, if any, at concentrations always lower than limits admitted by the EP 9.0 specifications. Formulations **SF1** and **SF2** contained impurities at detectable levels, but within EP 9.0 specifications. Formulation **SF3**, instead, did not contain any volatile organic impurities at detectable levels in the used experimental conditions. In particular, formulation **SF1** showed the presence of only one kind of impurities, *i.e.*, EP 9.0 impurity **B**, and its concentrations, in both analyzed batches, are within EP limit (100 ppm) and with low variation between samples. On the other hand, various impurities were found in all examined samples of formulation **SF2**. These impurities, which may have originated in the production process, were present in concentrations invariably within EP 9.0 limits. Different **SF2** batches were found to contain impurities with different structures, whose clinical relevance remains to be established. In analyzed batches, the content in sevoflurane, the active principle, is > 99.995% for **SF1**, > 99.99% for **SF2**, and > 99.9995% for **SF3**. Although processes for producing marketed sevoflurane formulations are trade secrets, the chemical nature of identified impurities **B**, **D**, and **E** may give indications on the adopted synthetic protocols, possibly suggesting they are different.

Finally, F^- ion concentrations were below 0.1 mg/L in all measured samples, consistent with the statement that no degradation occurred and with the fact that levels of volatile impurities are largely independent of the adopted storage conditions and are not substantially increased by accelerated ageing conditions. Water, a simple and convenient inhibitor of Lewis acids which may catalyze sevoflurane decomposition, is present in all the analyzed samples. Its levels are different in batches of different manufacturers and vary as a function of the storage conditions.

4. Experimental

4.1. Sevoflurane formulations

The analyzed Sevoflurane formulations (SF) were from Baxter (**SF1**), Piramal (**SF2**), and Abbvie (**SF3**, SEVOrane). **SF1** is commercialized in 250 mL bottles of epoxy-phenolic resin lined aluminum, **SF2** in 250 mL glass bottles, and **SF3** in 250 mL plastic (polyethylene naphthalate, PEN) bottles (Fig. S1). We analyzed two different batches for each producer, namely, batches no.s A17B28A30 (**batch-1**) and A18D13A30 (**batch-2**) for **SF1**, S0017A07 (**batch-1**) and S2957L13 (**batch-2**) for **SF2**, and 6066815 (**batch-1**) and 1091272 (**batch-2**) for **SF3**. Any analysis was performed by sampling freshly opened bottles stored under the ***std*** or ***aa*** conditions described above.

4.2. Sevoflurane related compounds

Commercial samples of Sevoflurane related compounds **A**, **B** (purity > 98%), and **C** (purity ≥ 99%) were obtained from Toronto Research Chemicals, TCI, and Sigma Aldrich, respectively. Chlorosevo ether (**CSE**, purity > 95%) was obtained from Fluorochem. All the above mentioned compounds were used as standards for calibration after proper dissolution in ethylene chloride (Fluka) as solvent.

4.3. Storage conditions

All samples were stored closed in their original shipping packaging under normal temperature, RH, and lighting conditions (***std***, average temperature T = 24 °C and average RH = 36%) in a laboratory fume hood, and under accelerated ageing conditions (***aa***, T = 40 °C and RH = 75%) in a Memmert Humidity Chamber (HCP 108, Fig. S2).

4.4. Gas Chromatography-Mass Spectrometry (GC–MS) analysis

Sevoflurane samples were analyzed by GC–MS in order to evaluate and quantify the impurities specified by European Pharmacopoeia 9.0. Chromatographic separation was obtained with a Perkin Elmer Clarus 500 GC system and a Thermo-Fisher Scientific™ TraceGOLD™ TG-1301MS 30 m × 0.25 mm I.D. × 0.25 µm film capillary column. Additional details of instrument parameters are reported below. The use of a mass spectrometer as the detector in gas chromatography allows to increase considerably the sensitivity of the measurements, overcoming the issues associated with Flame Ionization Detectors (FID), less sensitive in the detection of fluorinated substances. Total ion current was used as quantification method. Reported uncertainty values are estimated based on standard deviations of multiple analyses.

Commercial samples of pure Sevoflurane-related compounds **A**, **B**, and **C** were used as standards for calibration after dissolution in ethylene chloride as solvent. Sevoflurane samples were, instead, analyzed as pure materials without further dilutions, in order to maximize the sensitivity of the method and possibly detect impurities at very low concentrations. Figures S3-5 report calibration curves for compounds **A**, **B**, and **C**, respectively.

For both calibration standards and samples analyses, the experimental conditions detailed in Table 5 were used. Column specifications:

Column provider: Fisher Scientific

Column length, l = 30 m;

Column diameter, Ø = 0.25 mm;

Type of stationary phase: Poly[cyanopropyl)(phenyl)][dimethyl] siloxane (6%);

Stationary phase film thickness = 0.25 µm;

Stationary phase polarity: Low to mid-polarity phase.

The organic impurity contents were measured making at least three replicas for each sample.

Retention times (min.) of the analyzed compounds are: **A** = 5.92, **B** = 5.85, **C** = 8.15, **D** = 7.76, **E** = 7.96, **F** = 7.00.

Retention time for sevoflurane falls within the window between 6.0 and 6.9 min. In the analysis of the samples, the filament has been turned off in this time window to avoid sevoflurane acquisition. None of the standard peaks overlaps with the sevoflurane peak.

GC–MS chromatograms of some of the performed analyses are reported in Figures S6-12.

Table 5
Experimental conditions for the GC–MS analysis.

	Time (min)	Temperature (°C)	Heating rate (°C/min)	Flow (mL/min)
Column	0-6	32		
	6-23	32→200	20	
Injection port (PSS)			200	
Split				10
He flow				0.8

4.5. High-Resolution Mass Spectrometry (HRMS)

A Q Exactive GC–MS/MS Orbitrap mass spectrometer was used. Sample injection into a hot split/splitless injector (200 °C) was performed using a Thermo Scientific™ TriPlus™ RSH™ autosampler, and chromatographic separation was obtained with a Thermo Scientific™ TRACE™ 1310 GC system and a Thermo Scientific™ TraceGOLD™, TG-624SilMS 30 m × 0.25 mm I.D. × 1.4 µm film capillary column.

Additional details of instrument parameters are reported below.

- Liquid injection, split liner
- Oven: 32 °C for 6 min, 20 °C/min to 200 °C for 10 min
- Flow: helium constant flow 0.8 mL/min
- SSL 200 °C split flow 10 mL/min
- Injection volume 1 µl
- Ion source temperature 200 °C (T 150 °C for PCI)
- Transfer line temperature 200 °C
- Acquisition FullScan 33–500 amu
- Resolution power 60,000
- 70, 40, 20, and 10 eV ionizing energies were tried, Fig. 4 spectra were obtained by using 70 eV ionizing energy.

Original plots of part of the spectra are reported in Figures S14-15.

4.6. Ion–Exchange Chromatography analysis

Sevoflurane samples were analyzed by Ion Exchange Chromatography (IC) using a Thermo Fischer Dionex Acquion instrument against a standard containing known concentrations of seven inorganic anions (Seven Anions Dionex). Calibration curves (Figures S16-18) made with solutions containing known concentrations of F^-, Cl^-, and NO_3^- anions were used to determine the content of such anions in sevoflurane samples.

Anions present in the sevoflurane samples were first extracted with a 0.09 M sodium carbonate aqueous solution (Na_2CO_3, chromatographic eluent). 5 mL of Na_2CO_3 solution were mixed with 5 mL of Sevoflurane. The mixture was stirred for 5 min, then the sample was allowed to stand until complete phase separation. The aqueous phase was analyzed by IC.

4.7. Karl Fischer titrations: determination of water content

The amount of water present in Sevoflurane samples was estimated by Karl Fischer titrations using a Karl Fischer Metrohm instrument model 870 K F Titrino plus. The titrant (Hydranal Composite 5k - Fluka) was initially standardized by titrating known amounts of water (10 µL). Calibration was performed at the beginning of the test by injecting 5 aliquots of demineralized water (10 µL each). The water present in sevoflurane samples (from 1 to 5 mL depending on the water content) was subsequently titrated, using methanol HPLC grade (H_2O < 0.0015% - Fisher Chem) as solvent and making at least 5 replicas for each sample in order to provide a more accurate standard deviation on the data. Reported uncertainty values are estimated based on standard deviations of the 5 replicas.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jfluchem.2019.109363.

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