ORIGINAL ARTICLE

Stability study and development of the validated infrared spectrometric method for quantitative analysis of sevoflurane compared with the gas chromatographic method

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ABSTRACT

Sevoflurane, also called fluoromethyl ether, is an inhalation anesthetic agent used to initiate and maintain general anesthesia for adults and pediatric patients during surgical procedures. Several analytical methods have previously been applied to follow the properties and quality of sevoflurane, including mass spectrometry and gas chromatography methods. These methods are practically tedious and need sophisticated apparatus. In the present work, an attenuated total reflectance–Fourier transform infrared (ATR-FTIR) spectrometric method was used for the quantitative determination of sevoflurane which is characterized as a fast, accurate, and available technique for most pharmaceutical laboratories, besides the gas chromatographic method which is the most suitable for the detection of impurities. Sevoflurane is a liquid and it is applied directly on the glass top of the ATR-FTIR either as a concentrated solution or diluted with hexane as a diluent, which did not interfere with sample determination within the specified wavelength range of the IR spectrum, particularly the wavelength of the ethereal group at 1200 cm^{-1}. This method can be applied to the identification test and quantitative assay of sevoflurane since it is validated for the precision, accuracy, reproducibility, and specificity in the analysis of sevoflurane as a pharmaceutical product. However, still, there is a need for a gas chromatographic method to detect the impurities and degradation products during the stability study of sevoflurane.

Key words: Attenuated total reflection infrared spectrometer, degradation products, gas chromatography, quantitative method, validation

INTRODUCTION

Sevoflurane is a fluorinated methyl isopropyl ether used as an inhalational anesthetic for both induction and maintaining general anesthesia during surgical procedures. Sevoflurane is reported as a part of its physical–chemical properties to be clear, colorless, volatile, nonflammable liquid, and slightly soluble in water. It is sensitive to light

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and high temperatures; therefore, it should be stored in airtight containers at a temperature of 20° to 25° and protected from light.[1]

Sevoflurane has become widely used compared with other inhalational anesthetics as it is safe, nonpungent, and suitable for adults and children.[2,3] However, total intravenous anesthesia and establishment of venous access before inhalation anesthesia are particularly suitable for pediatric patients.[4] Such a clinical report indicated the use of sevoflurane to maintain the anesthesia with applications of different general anesthetic agents in otolaryngology surgery.[5] Other scientists compared the effects of sevoflurane with another inhalation anesthetic agent (propofol) and proved that sevoflurane has a good anesthetic-maintaining effect and a good effect on endogenous nitrogen oxide metabolism in patients under laparoscopic surgery.[6] Mapelli *et al*. have studied the effects of the general anesthetic sevoflurane on neurotransmission and reported that sevoflurane shapes neuronal communication without silencing the neural circuits.[7]

The chemical structure of sevoflurane is 1,1,1,3,3,3-hexafluoro-2-(fluoromethoxy) propane which is synthesized by different methods as pure liquid with expected industrial impurities. Fluoromethyl 2,2-difluoro-1-(trifluoromethyl) vinyl ether (compound A) may be produced from the degradation of sevoflurane by dehydrofluorination and should not exceed 25 ppm.[8]

Administration of sevoflurane for inhalation as an anesthetic is usually applied in a closed system of the vaporizing apparatus, resulting in CO_2 released during the breathing process, and therefore, CO_2 absorbents should be used in the inhalation system. Most of the CO_2-absorbents consist of a strong base including sodium hydroxide and calcium oxide or a concentrated solution of potassium hydroxide (such as soda lime and baralyme), these strong bases, in turn, enhance the formation of (compound A) as a degradation product of sevoflurane which has been proved to be nephrotoxic**.** Several studies of the chemical breakdown of sevoflurane to its related compound A have been discussed using different types of CO_2 absorbents.[9-12] It has been reported that CO_2-absorbent containing potassium hydroxide produces more concentration of compound A from sevoflurane in clinical practice.[13] However, all the volatile anesthetics agents (such as halothane, enflurane, isoflurane, and sevoflurane) undergo degradation on exposure to soda lime absorbents, almost all degraded compounds of these volatile anesthetics resulted from the elimination of hydrofluoride from adjacent carbon atoms of their molecules.[14] Feldman *et al*. made a new formula for CO_2-absorbent by the elimination of potassium hydroxide with the use of sodium hydroxide in low concentrations, <2%, the compound A formation becomes no longer a concern, and anesthetic inhalation of sevoflurane becomes relatively safe, as long the required storage conditions of the product and periodic tests are maintained.[15]

The most current method of analysis used in the determination of sevoflurane and its impurities is the gas chromatography (GC) technique using a capillary column with a flame ionization detector or combined with a mass spectrometer. This technique allows us to analyze sevoflurane quantitatively in an aqueous solution or in biological fluid, blood, and urine.[16-18] GC has also been used in different stability studies of sevoflurane to detect the degradation products, on the other hand, the GC method is a rather complicated instrument that needs time in operation and maintenance, therefore, an attempt to use another simple technique. In this work, the attenuated total reflectance infrared (ATR-IR)-spectrometer was used particularly for qualitative and quantitative analysis of formulated sevoflurane. The novel technique, the ATR-IR instrument nowadays makes the analysis fast, simple, and suitable for quantitative tests of liquid and solid samples. In this work, the ATR-IR was used to measure the quantity of sevoflurane liquid by comparing it with the original product at the specific wavelength of the ethereal functional group. This method was validated for precision, accuracy, reproducibility, and specificity as a general requirement in evaluating efficiency. The stability tests of sevoflurane of stored products were also carried out using the ATR-IR method of analysis and the results were compared with the gas chromatographic data.

MATERIALS AND METHODS

Materials; sevoflurane USP liquid (Shanghai Hengrui Pharmaceutical CO. Ltd.), hexane liquid, potassium hydroxide granules, sodium fluoride, all (Reagent Grades). Apparatus; GC (Shimadzu Co.), ATR-IR instrument (Bruker Co.), pH-meter with fluoride electrode, potentiometer titration apparatus (Safa Co. Quality Control Lab., Baghdad).

Experimental work

Attenuated total reflectance-infrared procedure

The ATR IR spectrometer is a portable instrument used for qualitative and quantitative analysis of materials in the case of powder or liquid, showing their IR spectrum in a simple application. It is based on passing the ray of IR light through a hemispherical crystal of a high refractive index material such as germanium, which reflects off the internal surfaces in contact with the sample under test.

In the case of sevoflurane liquid, a drop of the sample was placed on the ATR crystal after zeroing the apparatus with air. The IR spectrum of the sample appeared on the screen, and the analysis did not take more than 2 min. On the other hand, the software of the instrument carried out the measurement of the peaks according to the demand of either the absorption or reflectance intensity at the indicated wavelength of the liquid sample; sevoflurane is

a volatile liquid; therefore, the experiment was operated at low temperatures of environment between. The ATR-IR instrument is usually, supplied by library software to show directly the identification result of the sample.

For the quantitative test of sevoflurane, the absorbance of a sample should be compared with the absorbance reading of standard material of the same concentration following the absorbance of the response peak at a specific wavelength (about 1200 cm^{-1}), which contributed to the ethereal group of sevoflurane sample [Figure 1]. To determine the linearity of the relationship between peaks absorbances and the different dilutions of sevoflurane which were prepared using hexane diluent, as it has no IR peak response at the wavelength range of interest between 800 and 1300 cm^{-1} [Figure 2]. Hence the IR spectrum of pure sevoflurane was shown in Figure 3.

Validation of attenuated total reflectance-infrared for quantitative determination

The newly developed ATR-IR spectrometric method was validated for the quantitative determination of sevoflurane by carrying out the stated validation tests in US pharmacopeia protocol and some reported scientific works.[19,20]

1. Precision test: Different concentrations of sevoflurane liquid were prepared by transferring (1, 2, 3, 4, and 5 mL) accurately measured sevoflurane to five separated volumetric flasks of 10 mL size, and the volume of each was completed using hexane liquid as diluent. The ATR-IR determinations were carried out to establish the relationship between the different dilutions of sevoflurane and their absorbances, [Figure 4]
2. Accuracy test: 4 and 2 mL of sevoflurane solutions were transferred to two separated volumetric flasks of 10 mL size and diluted to volume with hexane then determined by ATR-IR apparatus, the readings of the resulted absorbances complied with readings on the standard curve of linearity in this method
3. Reproducibility test: Five mL of sevoflurane solution was measured accurately and transferred to a volumetric flask (size 10 mL), then the volume was completed using hexane as a diluent. The absorbance of the prepared mixture was determined directly by ATR-IR, and the absorbance of the IR spectrum at a wavelength (1204 cm^{-1}) was recorded. This procedure was repeated five times for the same prepared solution and the absorbance readings of the peak responses were recorded with a calculated relative standard deviation (RSD) of 0.57% (the USP limit NMT 2%)
4. Specificity test; as the determination of sevoflurane is carried out at a selective wavelength of IR analysis which particularly resembles the ethereal functional group of sevoflurane; therefore, this method indicated its specificity for sevoflurane liquid and there was no interference from any other materials.

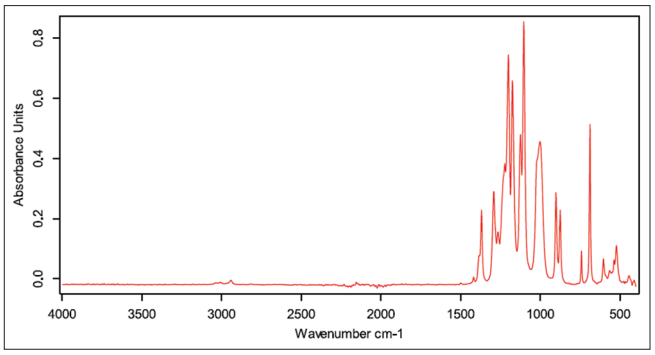

Figure 1: The infrared (IR)-spectrum of sevoflurane by attenuated total reflectance-IR instrument

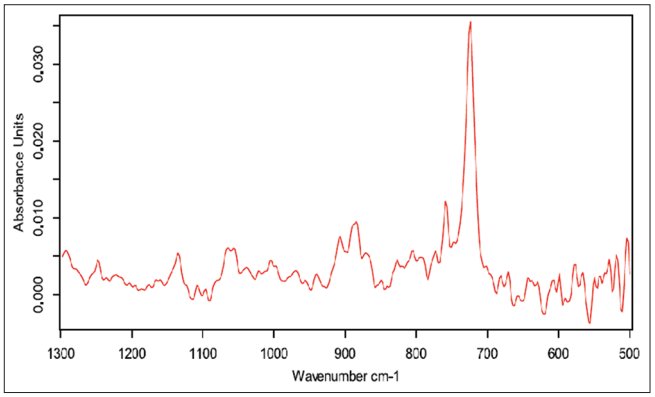

Figure 2: Infrared-spectrum of hexane; indicating the blank absorbance in WL range between 800 and 1300 cm^{-1}

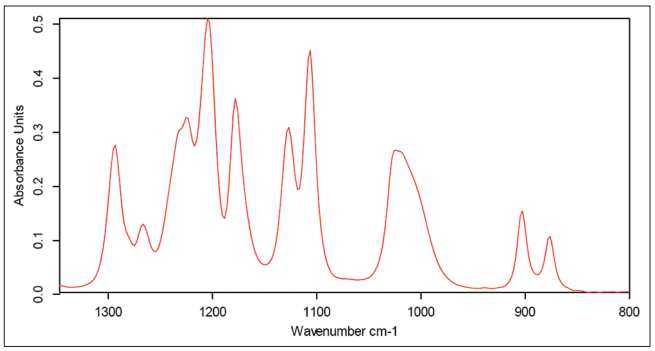

Figure 3: Sevoflurane 0.5% v/v in Hexane diluent shows a peak of absorbance at 1204.2 cm^{-1}

Gas chromatographic method procedure

The used gas chromatographic conditions in this work consisted of the following; Column; fused-silica capillary column, 30 m, ID; 0.53 mm, coated film, with liquid phase G43 thickness 3.0 µm. Detector; FID detector, operated at temperature 250.0°C, Carrier gas; Helium, injection volume; 2 µL.

With the application of this GC method, the impurities of sevoflurane become easy to detect and can be tested quantitatively. The USP monograph of sevoflurane mentioned the impurities as sevoflurane-related compounds

A, B, and C and gave a table for their relative retention times to the GC peak of sevoflurane.

In the GC procedure for sevoflurane, the pure sample was injected as 2 µL in a column operated under the prescribed chromatographic conditions above, and the percentage of sevoflurane was determined by subtraction of the peak areas of the impurities from 100. In the case of pure and freshly manufactured sevoflurane, the impurities will be very minute or negligible, and the percentage of sevoflurane is between 99.97% and 100%, this is calculated by the software of the apparatus, [Figure 5]. However, in the presence of detected impurity as compound A, the percent of sevoflurane was calculated as 97.08%, [Figure 6].

The assay procedure of sevoflurane was also carried out by the GC method, in which dimethoxy methane was used as the internal standard and ethylene dichloride as a diluent [Figure 7].

Stability study

Several scientific reports have revealed the stability data of sevoflurane, particularly in an evaporated state during operation, and discussed the effect of alkali adsorbent used in inhalation apparatus which leads to the degradation of sevoflurane and was reported to be of first-order kinetic.[21-25] Usually, the stability work includes periodic tests of several stored samples, therefore, the simplicity, accuracy, and rapid determination character of the ATR-IR method enabled this novel method to be the method of choice for stability analysis. In this work, samples of sevoflurane in their commercial product packed in amber glass containers were initially analyzed and then stored in stability chambers at two types of storage conditions as two groups. The first group of samples was stored at accelerated conditions, where the stability Chamber was fixed at 40°C, 75% relative humidity (RH), for 6 months. The second group was stored at 30°C, 65% RH, for 12 months. Samples of these groups were subjected to full analysis to investigate any pharmaceutical or chemical changes that might have occurred in different periods of storage. All the resulting marks are postulated in stability Tables 1 and 2, taking into consideration that the percentages of sevoflurane were determined by the ATR-IR spectrometric method and by the GC method.

RESULTS AND DISCUSSION

Examination of the IR spectrum of pure sevoflurane liquid by ATR-IR spectrometer complied with a reference solution of sevoflurane and the peak of absorbance at 1200.4 cm^{-1}, indicating the response of the ethereal group of sevoflurane [Figure 1].

For the selection of proper diluent, running pure hexane in an ATR-IR spectrometer did not show any absorbance

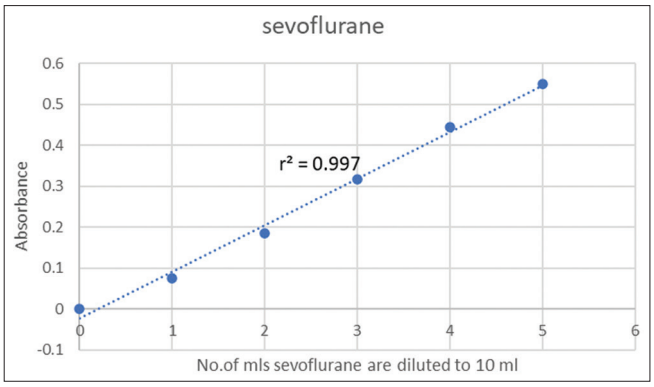

Figure 4: The linearity of quantitative determination of sevoflurane by ATR-infrared-Spectrometric method, eq. ($y = 0.114x - 0.02$)

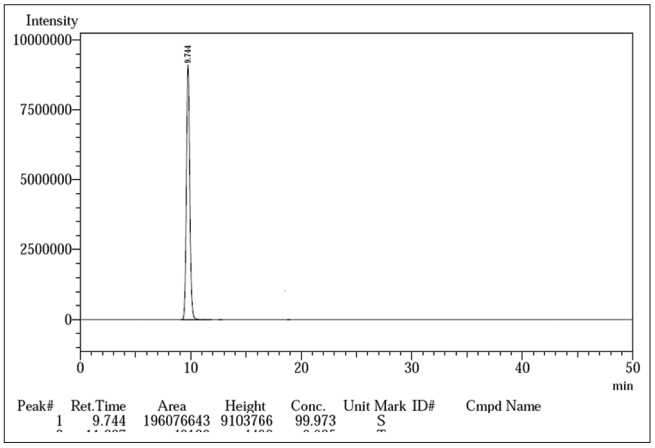

Figure 5: Gas chromatography-chromatogram of pure sevoflurane liquid (Rt; 9.7 min)

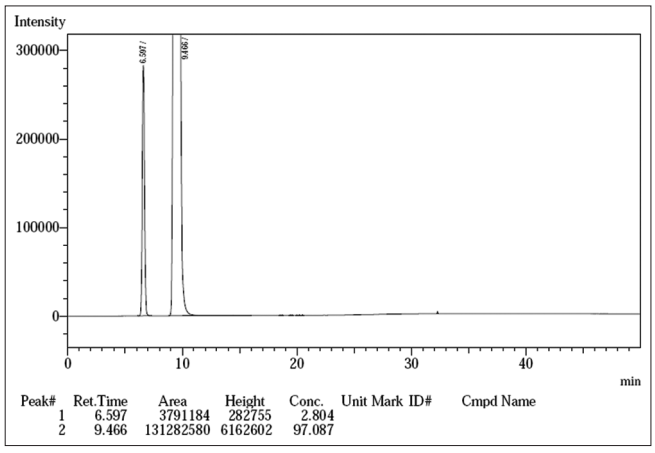

Figure 6: Gas chromatography-chromatogram of sevoflurane forms compound A (Rt; 6.7) on exposure to alkaline stress for 24 h

peak in the range between 800 and 1,300 cm^{-1}, this blank area makes hexane very suitable diluent in measuring the concentration of sevoflurane [Figure 2].

An experiment of quantitative analysis by ATR-IR was performed by measuring the ATR-IR spectrum of the diluted solution of sevoflurane with hexane (1:1), which showed a

peak at (1204 cm^{-1}) with absorbance intensity of (0.5132). The percentage of sevoflurane was calculated in relation to the absorbance reading of the standard solution of the original sevoflurane 0.5133 which resulted in (99.98%).

For the validation test of quantitative analysis of sevoflurane by ATR-IR method, the obtained data construct a straight-line relationship between sevoflurane concentrations and their IR absorbances [Figure 4]. This result indicates the precision of the ATR-IR method in quantitative analysis.

In addition, the accuracy of the method was proved when two different solutions of sevoflurane of known concentrations showed exact values on the calculation of their peak absorbance using the linear equation obtained. The obtained absorbance readings for the same sample were as follows: 0.513, 0.516, 0.509, 0.517, and 0.516, respectively. The calculated RSD percent of these results was 0.57% (the normal USP limit NMT 2%), this indicates the reproducibility of the ATR-IR method in quantitative analysis. Finally, the ATR-IR method indicates its specificity, since the test result depends on the specific structural group of sevoflurane (ethereal group).

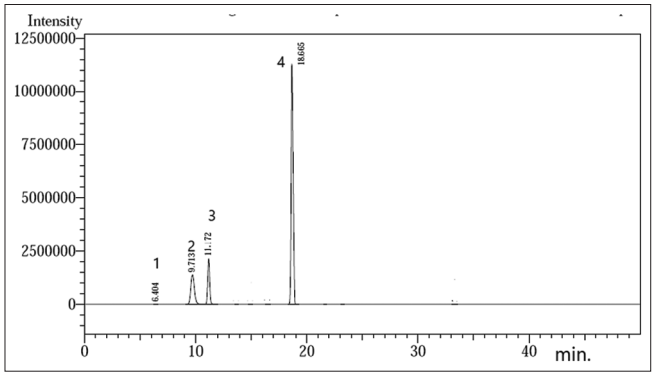

Figure 7: Gas chromatography-chromatogram of Sevoflurane in the presence of its degradation product (1); related compound A, (2) Sevoflurane, (3) internal standard, (4) solvent diluent; ethylene dichloride

Gas chromatographic method; the application of the gas chromatographic method in the analysis of sevoflurane proved good efficiency in qualitative and quantitative determination with the good ability to recognize the type of impurities and their quantities in products. The analysis of a studied pure sample of sevoflurane with the absence of other impurities showed that the percentage of sevoflurane is 99.973% [Figure 5]. The forced degraded sample in alkaline condition showed a peak at 6.5 min resembling the formation of related compound A with a percentage of the parent compound sevoflurane of 97.08%, [Figure 6]. This reduction in the concentration of sevoflurane has also been seen using the ATR-IR technique.

The results of the accelerated stability study of sevoflurane as finished products packed in amber glass bottles and stored for 6 months at (40°C and 75% RH) are illustrated in Table 1.

The results of the long-term stability study of sevoflurane in its finished products, packed in amber glass, and stored for 12 months at 30° C, 65% RH are illustrated in Table 2.

CONCLUSION

This work indicated that the new technique ATR-IR is precisely suitable for the quantitative determination of freshly prepared sevoflurane as a part of the quality control of the product to ensure its assay within the acceptable percentage, this technique is proven to be rapid, simple, and accurate with lower cost than the gas chromatographic instrument. On the other hand, the GC is the selective technique for the detection of degradation products in inhalational anesthetic agents, and therefore, both the ATR-IR and GC methods are considered stability-indicated methods for the analysis of sevoflurane. The studied samples of sevoflurane inhalational anesthetic agent were practically proved to be stable on the accelerated and long-term conditions of storage.

Table: 1: Accelerated stability study data of sevoflurane

Test	Specification	Initial	2nd month	4th month	6th month
Appearance	Clear colorless liquid	Clear and colorless	Clear and colorless	Clear and colorless	Clear and colorless
Identification	IR - spectrum complies with the reference	Comply with reference	Comply with reference	Comply with reference	Comply with reference
Refractive index	1.2745–1.2760 at 20°C	1.2755	1.2753	1.2753	1.2754
Alkalinity	No >0.6 mL of 0.01 N HCL solution require for neutralization	0.02 mL of 0.01 N HCL solution	0.02 mL of 0.01 N HCL solution	0.02 mL of 0.01 N HCL solution	0.02 mL of 0.01 N HCL solution
Non-volatile residue	Not >1 mg per 10 mL of sevoflurane	0.3 mg/10 mL	0.3 mg/10 mL	0.4 mg/10 mL	0.4 mg/10 mL
Water (%)	NMT 0.05	0.03	0.032	0.034	0.038
Fluoride (μg/mL)	NMT 2	0.04	0.04	0.04	0.04
Related compound A	ND	ND	ND	ND	ND
Assay percent	99.97~100.00 of C4H3F7O	99.99	99.99	99.99	99.99

ND: Not detected, IR: Infrared

Table 2: The stability study data of long-term storage

Test	Specification	Initial test	3rd month	6th month	9th month	12th month
Appearance	Clear and colorless	Clear and colorless	Clear and colorless	Clear and colorless	Clear and colorless	Clear and colorless
Identification	Comply with IR - spectrum of reference	Comply	Comply	Comply	Comply	Comply
Refractive index	1.2745–1.2756	1.2755	1.2753	1.2754	1.2754	1.2754
Alkalinity	NMT 0.6 mL of 0.01N HCL for neutralization	0.02 mL of 0.01 N HCL solution	0.02 mL of 0.01 N HCL solution	0.02 mL of 0.01 N HCL solution	0.02 mL of 0.01 N HCL solution	0.02 mL of 0.01 N HCL solution
Nonvolatile residue	NMT 1 mg per 10 mL sevoflurane	0.3 mg per 10 mL	0.32 mg per 10 mL	0.36 mg per 10 mL	0.38 mg per 10 mL	0.4 mg per 0 mL
Water (%)	NMT 0.05	0.03	0.034	0.037	0.039	0.04
Fluoride (μg/mL)	NMT 2	<0.2	< 0.2	<0.013	<0.013	<0.018
Related compound A	ND	ND	ND	ND	ND	ND
Assay (%)	99.97～100.00 of C4H3F7O	99.99	99.99	99.99	99.99	99.99

NMT: Not more than, ND: Not detected, IR: Infrared

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Conflicts of interest
There are no conflicts of interest.

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