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Research paper

Pharmaceutical and analytical characterisation of (2*R*)-(3-amino-2-fluoropropyl)sulphinic acid, a $GABA_B$ receptor agonist

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Abstract

(2*R*)-(3-Amino-2-fluoropropyl)sulphinic acid (AFPSiA) is a potent $GABA_B$ agonist, which makes it a possible alternative in future GERD treatment. The degradation of AFPSiA was investigated to support the drug-development effort. The compound is too polar to be compatible with regular reversed-phase LC. Moreover, the compound and the degradation products cannot be detected by UV due to low absorption. Instead, the degradation of AFPSiA was followed by two different capillary electrophoresis methods with indirect UV detection and ^{1}H NMR and ^{19}F NMR. AFPSiA was very unstable in basic conditions and at temperatures above room temperature. The corresponding sulphonic acid and allylamine are formed via two separate degradation routes. Both these degradation products may cause unwanted side-effects *in vivo*. Aqueous solutions of AFPSiA were found to be more stable at pH between 1 and 3. It was suggested that AFPSiA should be stored frozen, preferably at −70 °C. In solid state, the compound can be stored at ambient conditions in closed vials with low relative humidity. A solid oral dosage form should be kept in a blister package.

Keywords: Gastroesophageal reflux disease; GABA; GERD; Capillary electrophoresis; Indirect UV detection; NMR; Allylamine

1. Introduction

Tonic contraction of the lower esophageal sphincter (LES) is the principal factor preventing the reflux of gastric contents into the esophagus. Transient LES relaxation (TLESR) is the major mechanism underlying reflux in normal subjects and patients with gastroesophageal reflux disease (GERD) [1]. Currently, the prevalence of esophagitis in the Western world is a few percent but the prevalence of frequent heartburn is 10 times higher. GERD is nowadays in most cases successfully treated with proton pump inhibitors, but a number of patients do not receive full symptomatic alleviation [2,3]. In many of these patients, reflux of bile and other non-acid contents from the stomach is believed to generate symptoms [4]. Since TLESR is the dominant cause of reflux, interventions aimed at inhibiting TLESRs may have a therapeutic role to play in GERD [5,6], particularly when acid secretion suppression is not sufficiently successful.

Control of reflux through pharmacological inhibiting of TLESR is a conceptually attractive approach to the treatment of GERD. A promising class of agents identified to date for the reduction of TLESR is γ-amino butyric acid receptor ($GABA_B$) agonists [7–11]. GABA (Fig. 1) is a major inhibitory neurotransmitter within the central nervous system, and $GABA_B$ receptors are present at many sites in both the central and enteric nervous system [12]. The skeletal muscle relaxant baclofen, the only $GABA_B$ agonist approved for clinical use, reduces TLESRs in dogs [8], ferrets [7], cats [10] and humans [9,11]. Data have emerged to show that baclofen also provides reduction in reflux of both acid and non-acid gastric contents and symptomatic relief in GERD patients [4,13].

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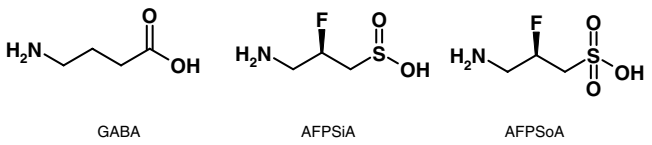

Fig. 1. Structures of GABA, AFPSiA and AFPSoA.

While being the preferred pharmacological option in the treatment of spasticity, baclofen may be less useful in GERD due to its side-effects from CNS. The need for novel $GABA_B$ agonists with an acceptable safety and tolerability in GERD is therefore obvious.

GABA analogues in which a sulphinic acid group replaces the carboxylic acid group of GABA have been evaluated. Such a compound, (2*R*)-(3-amino-2-fluoropropyl)sulphinic acid (AFPSiA, Fig. 1), was initially evaluated with respect to a pharmaceutical point of view in the present report. Since AFPSiA is potent in reducing TLESR, it is a possible new drug in GERD treatment [14]. In the current work, the stability of the compound in different pH buffer systems and at elevated temperature conditions was investigated. In addition, stability in solid state and in formulations for oral and intravenous (iv) administration was evaluated. Since AFPSiA is very hydrophilic, with low molecular weight and no obvious cromophore in the molecule, specific attention have to be considered when developing analytical methods for identifying and quantifying the compound and its degradation products.

2. Materials and methods

2.1. Compound

AFPSiA is obtained from AstraZeneca. The substance is a crystalline compound with a melting point of 130–140 °C and a molecular weight of 141.2 g/mol. The compound takes up about 2.5% water at 80% RH (relative humidity) and is classified as hygroscopic (according to EP Technical guide, 1999). The compound has two pK_a values of <2 (the sulphinic acid) and 8.5 (the amine). Due to the fact that the compound is a zwitterion in aqueous solution, the molecule is highly polar (an estimated negative octanol/water partition coefficient, log K_D). This leads to high aqueous solubility (>200 mg/ml) in the *in vivo* pH range (pH 1–8).

2.2. Capillary electrophoresis (CE) and indirect UV detection for the bulk substance

A HP^{3D}CE system (Agilent, Waldbronn, Germany), equipped with a photodiode array detector, was used for the CE analysis of the substance. Bare fused silica capillaries (Polymicro Technology, Phonex, AZ, USA) of 50 μm ID and 48.5 cm total length (40 cm effective length) were used for the analysis. Indirect UV detection was performed with an analytical wavelength at 350 nm with reference wavelength at 210 nm [15–17]. Separation voltage was at 5 kV with negative polarity and the capillary was kept at 15 °C under separation. The sample solution was injected at 25 mbar under 10 s by the hydrodynamic mode.

A commercial background electrolyte Basic Anion Buffer for HPCE (Agilent, Waldbronn, Germany) was used after having been mixed with 0.01 mol/L NaOH (7:3). The pH of the electrolyte solution was at 12. No further optimisation was performed with respect of the electrolyte composition.

With the present method, it was possible to separate impurities, e.g. AFPSoA (a sulphonic acid of AFPSiA, i.e. an oxidation product, Fig. 1) from the main peak. Besides, other anions such as Cl^-, Br^-, I^-, SO_4^{2-}, PO_4^{3-} (appears from HPO_4^{2-} and $H_2PO_4^-$ at the present pH), formate ion, citrate ions, acetate ion, SO_4^{2-}, $S_2O_7^{2-}$, $S_2O_3^{2-}$, SO_3^{2-}, NO_3^-, PO_2^{3-} (after adding $H_2PO_2^-$), $B_4O_7^{2-}$, CO_3^{2-} (converted from HCO_3^- at the present pH) and SiO_3^{2-} were observed in the electropherograms. The ions were identified in spiked sample solutions.

Two milligram per millilitre of the active substance was formulated in aqueous solutions and used as sample solutions without further treatment.

The repeatability (RSD%, $n = 6$) for the method was less than 1% for AFPSiA while a linear response could be observed between 0.4 and 3.0 mg/ml, with a correlation coefficient value at 0.998.

2.3. Capillary electrophoresis (CE) and indirect UV detection for the formulation

The CE experiments were performed on a P/ACE MDQ capillary electrophoresis system (Beckman Coulter, Fullerton, CA, USA) equipped with a photodiode array detector. Bare fused silica capillaries (Polymicro Technology, Phonex, AZ, USA) of 50 μm ID and 50 cm total length (40 cm effective length) were used for the separation. All separations were carried out with a separation voltage at −15 kV (negative polarity). The sample solution was injected by hydrodynamic mode under 40 s at 0.3 psi. The analytical wavelength was set to 216 nm for indirect detection without employing any reference wavelength. The capillary was kept at 16 °C during the whole analysis process. Before each analysis, the capillary was washed with 0.1 M sodium hydroxide followed by 1 mM *N*-cetyltrimethyl-ammonium bromide (CTAB) to reverse the direction of the electroosmotic flow.

The background electrolyte was composed of 50 mM L-histidine, 0.1 mM CTAB and 30% v/v ethanol. The pH was adjusted with 0.1 M sodium hydroxide to 9.5, which gave the best peak form for AFPSiA. The L-histidine works as both a UV-absorbing agent and a buffer component. The concentration of L-histidine was a result of compromise between striving after higher signal and lower current. Since no extra co-ion was introduced as the UV-absorbing agent, the probability to be disturbed by system peaks could be reduced.

0.5 mg/ml of the active substance was formulated in 9 mg/ml NaCl and used as sample solutions without further treatment.

The existence of the "high" concentration of sodium chloride gives the CE analytical method a challenge. Normally, the background electrolyte should have a higher or similar ionic strength. That means the electric current generated during the separation would be higher which leads to increasing heat generation. Therefore the choice of the background electrolyte composition became a delicate balance between stability and separation performance. This is why there were two different CE methods with similar analytical principle developed for bulk substance analysis and for formulation analysis. While the sample solutions for the bulk substance analysis are a pure solution of the bulk substance with its degradation products (see Section 2.2), the sample solution for formulation analysis had always problem with sample matrix disturbance. Therefore the CE method suggested for bulk substance analysis had high separation capacity and thereby could offer higher selectivity, while suggested method for formulations is compromising selectivity to be able to tolerate a higher ionic strength of the sample solution. A higher electric field was also applied for the method for formulations to speed up the analysis, since the method was used to cope with a large amount of samples during safety assessment studies. No remarkable Joule heating effect was observed.

Typical electropherograms for formulation sample and bulk substance sample are shown in Fig. 2.

The CE method for formulation samples was validated with respect to selectivity, linearity, repeatability and accuracy according to an internal Standard Operating Procedure (SOP) for validation of analytical methods. The linearity was tested between 0.1 and 0.7 mg/ml, a correlation coefficient (r^2) over 0.9999 was obtained. The repeatability of six injections of standard solution at 0.5 mg/ml AFPSiA was, expressed as RSD of corrected peak area and of relative migration time, 1.91% respective 0.09%. The accuracy obtained was between 98.5% and 101.5%.

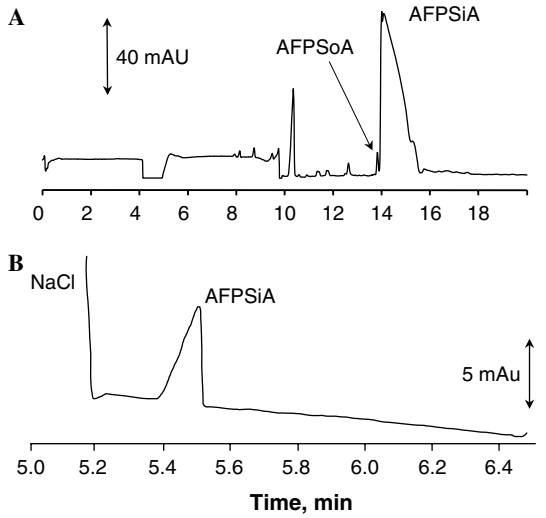

Fig. 2. Typical electropherograms for a bulk substance sample (A) and a formulation sample (B). In (A), peaks from AFPSiA and AFPSoA and small peaks originating from common ions existing in the background electrolytes appear. All peaks were spiked and identified (data not shown). The method for the bulk substance analysis had higher separation capacity and thereby could offer higher selectivity, while the method for formulations (B) needs to tolerate higher ionic strengths. Detection of AFPSoA formation with the formulation method is illustrated in Fig. 3. Further experimental conditions are given in Sections 2.2 and 2.3.

2.4. ^{1}H NMR

The ^{1}H NMR measurements were carried out on a Varian 500 MHz Inova instrument operating at 499.545 MHz. The NMR instrument was equipped with a 5 mm inverse detection probe. The samples were dissolved in D_2O and the concentration was about 1% (w/w). The temperature used was 25 °C. A typical ^{1}H NMR measurement was carried out by using a flip angle of 45° with an acquisition time of 4 s and a delay time of 4 s. The spectral width was at least between 0 and 8 ppm referring to the solvent peak of D_2O (4.80 ppm) and 16 transients were obtained. A zero filling of at least twofold in size was applied and a line broadening factor of 0.3 Hz was used. The spectra were phased and baseline corrected prior to integration.

2.5. ^{19}F NMR

The ^{19}F NMR measurements were carried out on a Varian 600 MHz Inova instrument operating at 564.511 MHz. The NMR instrument was equipped with a Varian triple resonance 5 mm probe with the proton channel tuned to fluorine. The samples were dissolved in D_2O and the concentration was about 1% (w/w). The temperature used was 25 °C. The ^{19}F NMR measurements were commonly carried out by using a flip angle of 45° with an acquisition time of 4 s and a delay time of 4 s. The spectral width was varied between 28 and 150 ppm depending on the type of ^{19}F NMR measurement. The frequency is set with reference to the deuterium lock. Different amounts of transients were obtained depending on the purpose of experiment. A zero filling of at least twofold in size was applied and a line broadening factor of 0.3 Hz was used. The spectra were phased and baseline corrected prior to integration.

3. Results and discussion

3.1. Stability at different pH and different temperatures

In the development of pharmaceutical dosage forms, one of the persistent challenges is assuring acceptable stability, i.e. the storage time allowed before the content is too low or a degradation product in the dosage form achieves a sufficient level to represent a risk to the patient. Based on this time, the expiration date (shelf-life) of a product is determined. The allowable level of any given impurity will depend on the dose and its potential of toxicity. In the present tables, we have chosen to measure the drug loss to determine stability instead of following the separate peaks of product formation (Tables 1–3). It is of high importance to identify any instability in pharmaceuti-

Table 1
Stability of AFPSiA in aqueous solutions

Temperature/solvent	Day 1 (%)	Day 3 (%)	Day 7 (%)
22 °C, pH 1	100	92	68
22 °C, pH 3	100	94	86
22 °C, pH 7	74	56	n.a.
22 °C, pH 12	56	n.a.	n.a.
22 °C/water, pH 5	85	53	34[a]
−20 °C/water	n.a.	101	100
50 °C/water	<20	n.a.	n.a.
80 °C/water	23	n.a.	n.a.

CE-content at day x versus content day 0 (%). Samples (2 mg/ml) were stored in transparent vials at ambient humidity. The buffers used were HCl (pH 1), citrate buffer (pH 3), phosphate buffer (pH 7) and carbonate buffer (pH 12).
n.a., not analysed.
[a] pH decreased about two pH units.

Table 2
Stability of AFPSiA (0.5 mg/ml) in 9 mg/ml NaCl solution

Time (days)	37 °C (%)	22 °C (%)	8 °C (%)	−20 °C (%)
0	99	99	99	99
1	65	96	n.a.	n.a.
3	9	87	98	n.a.
7	0	80	100	n.a.
14	n.a.	65	100	102
28	n.a.	3	91	97
56	n.a.	5	87	104

The results are listed as % of stated concentration.
n.a., not analysed.

Table 3
Solid state stability of AFPSiA

Climate	1 Week (%)	4 Weeks (%)	12 Weeks (%)
Laboratory, closed vial	100	100	100
40 °C/75% RH, open vial	98	<20	n.a.
50 °C, open vial	96	62	27
80 °C, open vial	<20	n.a.	n.a.

CE-content at day x versus content day 0 (%).
n.a., not analysed; RH, relative humidity.

cal formulations as early as possible in the development process. Polar zwitterionic compounds are as such not easily retained on conventional bonded stationary phases for reversed-phase chromatography. Morover, even if AFPSiA has a small UV absorption around 200 nm (caused by the sulphinic group), the low sensitivity is not useful for UV detection. MS-, amperometric- and ELS (evaporative light scattering) detection have been employed with limited success. For the present compound, capillary electrophoresis (CE) with indirect UV detection has been employed for analysis of content of AFPSiA, impurities from the process of manufacturing and degradation products. Since the indirect UV detection makes some common ions visible, it could raise the difficulty of identification of unknown degradation products. Several common anions and even anions released from the glass vials, such as silica and borate, could be detected in sample solutions with rising signals (see Section 2.2). A typical electropherogram shows a main peak of about 90% of the total area and a few percents originate from organic impurities or degradation products (Fig. 2A). Remaining 8–9% are common ions existing in the background electrolytes (see Section 2.2).

The stability of AFPSiA in solution was investigated at different pH and temperatures (Table 1). Degradation was observed at room temperature in solutions at pH between 1 and 12. The degradation was most pronounced at neutral and high pH. AFPSiA also degrades in water solutions at all temperatures examined, except at −20 °C, where the stability was found to be good for at least 3 days (see also Section 3.2). The pH of the degraded AFPSiA in unbuffered solutions decreased with time, indicating the formation of one or more acidic degradation product(s). Since no product for intravenous (iv) administration was planned, the degradation observed for the bulk compound in solutions was not considered to be a pharmaceutical issue (but may have possible toxicological consequences) as long as the compound is stable in its solid state form (however, see below).

3.2. Stability of formulations for iv and oral administration

Although the final aim of the project was to deliver a solid oral dosage form, at least one study was planned to be performed in human by iv dosing. This puts emphasis on the need to develop an iv dosage form for the substance. For iv studies the tonicity of the solution should be modified to be as close as possible to physiological tonicity, in order to minimize haemolysis and vessel damage. Saline is the most likely choice of tonicity modifier. A 9 mg/ml NaCl solution can dissolve >200 mg/ml of the substance. This formulation can probably be used in the toxicological programme as well as in the iv study(-ies) in human. With sodium chloride present, the same formulation can be used for iv and oral administration.

Solutions of AFPSiA showed no or minor degradation when stored in refrigerator (8 °C, stable at least ⩾2 weeks) and freezer (−20 °C) for 8 weeks (Table 2). However, a substantial degradation was observed at room temperature (22 °C). The degradation tendency, at room temperature, was further confirmed in a separate 1-week study, where AFPSiA was dissolved in distilled water. An even more pronounced degradation was observed at 37 °C, where just about 10% of the substance remained after 3 days (Fig. 3 and Table 2). During storage at 37 °C, freezer and refrigerator, the samples were protected from light.

Light exposure can induce chemical degradation. For light to be able to induce a chemical reaction, the light must be absorbed. In the present substance, there is no chromophore present which is supposed to absorb light significantly. Besides, the transmission of ambient light through glass is minimal below 300 nm. So keeping the solutions (in 9 mg/ml NaCl) protected from light did not improve the stability.

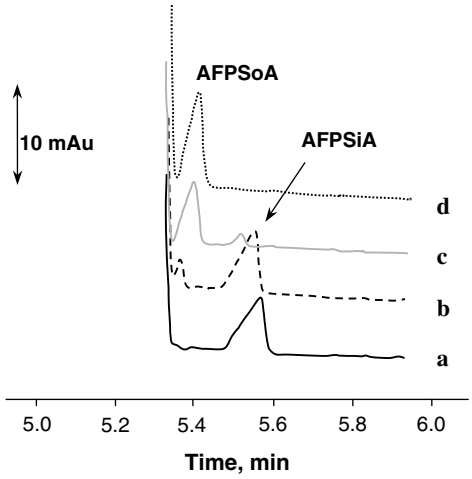

Fig. 3. Degradation of the AFPSiA and formation of AFPSoA during storage at 37 °C. The four electropherograms illustrate: an original electropherogram of AFPSiA with no AFPSoA present (a), the same formulation after 1-day storage at 37 °C (b), after 3-day storage at 37 °C (c) and after 7-day storage at 37 °C (d). Further experimental conditions are given in Section 2.3.

Oxidative degradation of pharmaceuticals caused by the presence of oxygen is a major concern. Nitrogen was used to maintain a non-oxidative environment. However, the presence of nitrogen did not improve the stability.

3.3. Stability in solid state

A requirement for the development of an oral solid dosage form is that the compound is possible to handle as bulk and that the solid state stability (both chemical and physical) is acceptable. Besides, it must be possible to manufacture and store a dosage form containing the compound.

AFPSiA is chemically stable at room temperature (Table 3). However, at stressed conditions degradation takes place and the compound was almost totally degraded at 80 °C in less than 1 week. Extensive degradation was also observed after 1 month at 40 °C/75% RH and at 50 °C.

After open storage for 1 month at 40 °C/75% RH, the compound became a transparent gel and ≈36% of the sample weight had evaporated. At 50 °C, during the same time period, the compound still had the appearance of a white powder, but about 4.5% of the weight had evaporated. For further evaluation of the degradation pathway(s), one or more complementary method(s) is needed (see below).

The compound was also directly compressed to a tablet without any excipients present and stored for 3 months in a sealed plastic package. The tablets of pure compound were both chemical (CE) and physical (X-ray powder diffraction) stable (data not shown).

3.4. NMR evaluation

In assessing the stability of drugs, the use of multiple methods can help in determining the mechanism of degradation. For the present compound, a complementary technique was employed to interpret the degradation mechanisms. During recent years the use of NMR has increased in popularity as an quantitative method to determine both assays and impurities due to its great sensitivity to small changes in molecular structure [18–20]. This sensitivity and the high resolution of modern instruments ensures that resolved peaks attributable to individual chemical species are observed. Separate peaks can be integrated independently from the other resonances in the NMR spectrum. Since the intensity of a signal is directly proportional to the number of nuclei evoking the signal, the intensities of NMR signals can be used for quantitative investigations. Moreover, there are a variety of NMR active isotopes suitable for measurements so that one does not necessarily have to rely on the ^{1}H spectrum for analysis. In the present paper, we have used ^{1}H and ^{19}F to identify and quantify the two main degradation products, allylamine and AFPSoA (an oxidation product), and the parent compound.

Employing ^{1}H NMR, allylamine was identified in a D_2O solution of AFPSiA (Fig. 4). At room temperature, about 1% (w/w) allylamine was found after 20 h and ≈10–15% (w/w) after 10 days. These findings explain a part of the fast degradation observed for AFPSiA. A possible degradation route is shown in Fig. 5. This mechanism explains the observed pH decrease in unbuffered solutions, which is mainly caused by the formation of HF (see Section 3.1). This mechanism also explains the evaporation of the bulk substance (see Section 3.3). Since this mechanism is the major degradation route, and the more crucial degradation to follow, it was decided to investigate this mechanism in more detail.

The rate constant k of the AFPSiA conversion to allylamine (Fig. 5) can be determined by the evaluation of the time-dependent decrease of intensity of the ^{1}H NMR signals in AFPSiA. By using one of the protons (2.71 ppm) in the methylene at position 3 in AFPSiA and assuming first order reaction kinetics we have,

$$I_t^{CH_2} = I_0^{CH_2} \exp(-kt), \quad (1)$$

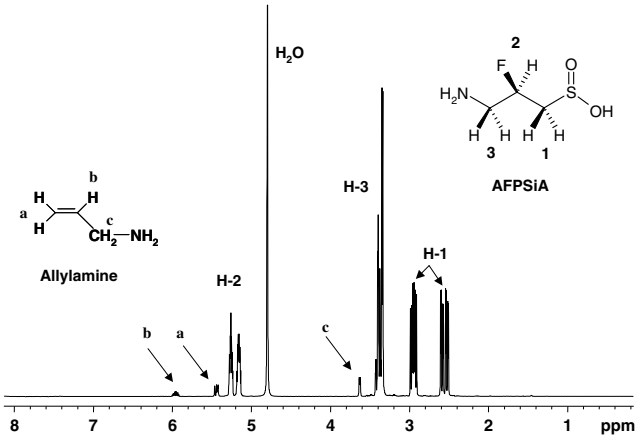

Fig. 4. ^{1}H NMR spectra showing AFPSiA and allylamine.

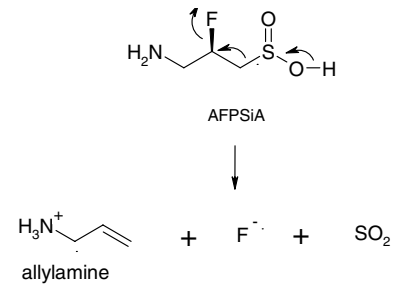

Fig. 5. The likely degradation route of AFPSiA, showing the formation of allylamine.

where $I_t^{CH_2}$ and $I_0^{CH_2}$ are the integral intensities of the methylene group at the reaction time t and at $t = 0$, respectively. The temperature dependence of the rate constants (Fig. 6) gives the Arrhenius plot shown in Fig. 7. The activation energy obtained for the conversion of AFPSiA to allylamine is 27.8 kcal/mol. Unfortunately, the observed three-carbon aliphatic alkylamine, allylamine, is known to induce acute myocardial necrosis due to coronary vasospasm and myocardial ischemia and cell injury ([21,22] and references therein).

AFPSiA contains a fluorine atom and makes ^{19}F NMR a choice as an impurity method. This method has been applied on AFPSiA and the degradation product AFPSoA, which has been detected and quantified (Fig. 8). From a recent study, it is obvious that a conversion of AFPSiA to AFPSoA occurs both in dogs and rats [14]. The formation of the oxidized metabolite does also induce a

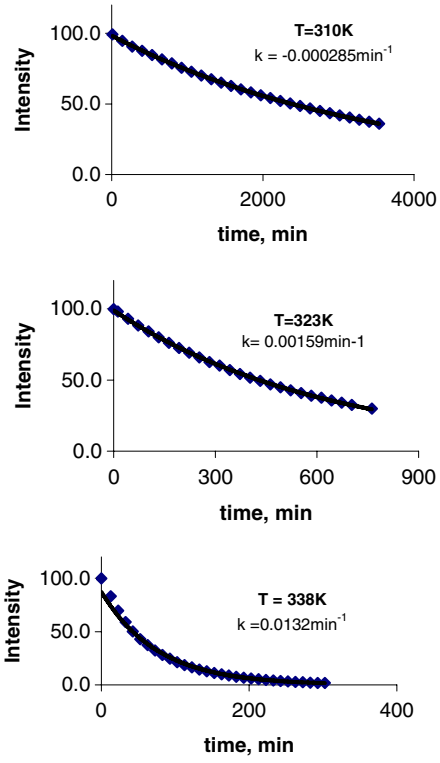

Fig. 6. Kinetics of the decomposition of AFPSiA to allylamine. The solid lines represent the fits to the experimental points by using Eq. (1) ($n = 1$).

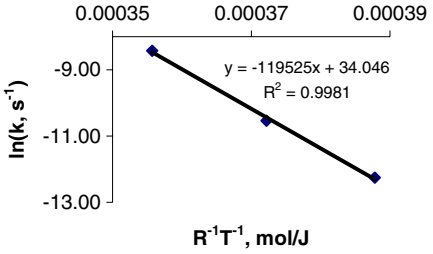

Fig. 7. Arrhenius plot for the decomposition of AFPSiA to allylamine.

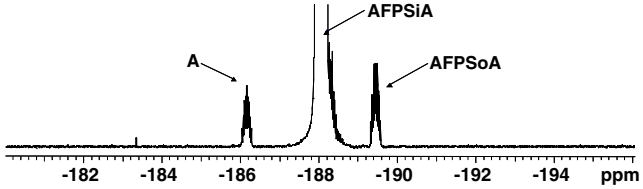

Fig. 8. The ^{19}F NMR spectrum showing AFPSiA, AFPSo and compound A, the latter containing the fragment: R–CH_2–CHF–CH_2–R_1. Peak areas are mol %.

dose-dependent hypothermia. After the highest dose tested (4 mmol/kg), the reduction in body temperature was so prominent that the mice had to be sacrificed 1 h after dosing on ethical grounds.

4. Conclusion

This work demonstrates that compounds like AFPSiA and its degradation products can be successfully analysed by a combination of two different CE approaches with indirect UV detection, ^1H NMR and ^{19}F NMR. The methods have proven to be well suited for routine application. The stability was studied with the present methods. AFPSiA was very unstable at basic conditions and at elevated temperatures. Then, the storage conditions must be carefully selected both for the bulk substance and the solutions. The formation of AFPSoA and allylamine can be controlled by keeping a liquid formulation of AFPSiA frozen and a solid dosage form of the compound in a blister package. However, since AFPSoA is formed *in vivo* [14], which also may be the case for allylamine, further development will be a difficult challenge.

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