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ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 44 (2007) 456–463
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Investigation of sorbic acid volatile degradation products in pharmaceutical formulations using static headspace gas chromatography

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Received 31 October 2006; received in revised form 12 January 2007; accepted 12 January 2007
Available online 19 January 2007

Abstract

An analytical method that allows simultaneous analysis of sorbic acid and its degradation products was developed using static headspace gas chromatography (HS-GC). AT-Aquawax-DA, the capillary column used, showed good selectivity and separation towards sorbic acid and its degradation products. Sorbic acid degradation was investigated in both acidic and aqueous media at room and elevated temperatures. In total 12 sorbic acid degradation products were found, 8 of which could be characterized. The method was investigated for its accuracy towards estimation of degradation products. Using the HS-GC method different batches of pharmaceutical preparations such as cold cream, cetomacrogol cream and vaseline were investigated for sorbic acid degradation products which were estimated by applying the standard addition method. Acetaldehyde was found to be the major degradation product. The other identified degradation products were: acetone; 2-methylfuran; crotonaldehyde; alfa-angelicalactone; 2-acetyl, 5-methylfuran; toluene and 2,5-dimethylfuran. Both mass spectrometeric (MS) and flame ionization detection (FID) were used. The qualitative investigation was done on HS-GC–MS and the quantitative work on HS-GC–FID.

Keywords: Sorbic acid; Degradation; Cold cream; Cetomacrogol cream; Vaseline; GC

1. Introduction

Sorbic acid is a widely used preservative that is found in many pharmaceutical formulations, especially in dermatological and cosmetic preparations. Sorbic acid and its salts have broad-spectrum activity against yeasts and molds. The pKa of sorbic acid is 4.76 and the working pH range is from 3 to 6.5. The extended preservation capacity up to pH 6.5 makes sorbic acid superior over other commonly used preservatives such as propionates and benzoates [1]. The amount used in most of the pharmaceutical preparations is 0.1% (w/w) and in some cases up to 0.15% (w/w). Despite its advantages as a preservative and a considerable stability in dry crystalline state, it is proved to be unstable in aqueous solution leading to several degradation products, thereby solutions turning to a yellow or brown color. Over 50 years, many research articles have been published about possible degradation of sorbic acid. Several degradation products from sorbic acid aqueous solutions on long standing and at different conditions were reported: acetaldehyde; malonaldehyde; acrolein; β-carboxyacrolein; acetone; crotonaldehyde (β-methylacrolein); 2-acetyl, 5-methylfuran and α-angelicalactone (4-hydroxy-3-pentenoic acid α-lactone). The major degradation path was proposed to be auto oxidation and the major degradation products (80%) due to this process were reported to be acetaldehyde and β-carboxyacrolein. It was also reported that the sorbic acid degradation products involve in chemical reactions and result in a change of organoleptic and nutritional properties of food preparations [1–12]. Some of the identified degradation products are known to be toxic: acetaldehyde is classified as possible carcinogen [13,14]; crotonaldehyde can be absorbed through the skin, is a potent respiratory, skin

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doi:10.1016/j.jpba.2007.01.020

and eye irritant [15] and genotoxic in the germ cells of mammals [16]. Acrolein, suspected carcinogen, may be a reproductive hazard and poisonous at ppm concentration levels [15]. Apart from their toxicity, these degradation products, often incompatible with acids, bases and amines, may be a threat for the stability of the formulation. The preservatives used in pharmaceuticals are strictly controlled by the respective regulatory organizations, preferring specific test methods for preservatives. There have been several methods published for the determination of the sorbic acid content in pharmaceutical and food preparations using different analytical techniques such as iodometric titration [17], polarimetry [18], UV–vis spectrometry [19–22], thin-layer chromatography [23], liquid chromatography [24–39], gas chromatography [40–49] and capillary electrophoresis [50–55]. For the investigation of the degradation products in the past, the analytical techniques used were: UV spectroscopy and thin-layer chromatography (TLC). So, no selective and sensitive analytical method to determine the degradation products of sorbic acid is available. From a pharmaceutical point of view, stringent regulatory specifications necessitate specific analytical tools for the characterization and determination of possible degradation products in a preparation during its shelf life.

In this study, GC was used as an analytical separation method. Both direct injection and static headspace (sHS) sampling modes were used to identify highly and semi volatile degradation products. In direct injection, the sample solution is injected directly into the heated GC injector. This way highly volatiles and semi volatiles can be analysed. The main disadvantage of this technique is that the non-volatiles from the injected sample will be retained either in the GC injector or in the column. These remnants may damage the column, degrade themselves and produce artefact peaks or influence the results of subsequent analyses. sHS sampling has great advantage in the analysis of highly volatiles in non-volatile sample matrices. In sHS the sample is enclosed in a gas tight sealed vial and is heated at a defined temperature. The high volatiles partition from the non-volatile sample matrix to the gas phase. After the equilibrium is attained between the gas phase and the sample matrix, part of the gas volume is injected into the GC. As the highly volatiles are concentrated in the headspace gas lower detection limits in comparison to the direct injection can be obtained. The major disadvantage of sHS is that the semi volatiles may not or less partition into the gas phase. Also, as this technique involves heating the sample matrix for a specified period of time, thermo liability of the sample matrix would be a limiting step in some analyses.

Initially, sorbic acid degradation was investigated in aqueous and acidic conditions using an optimised direct injection GC–MS method. The degradation was examined in both freshly prepared solutions and in solutions standing at room temperature for 3 months. Later, the method was optimised and validated using headspace sampling. Finally, different pharmaceutical preparations were investigated for possible volatile sorbic acid degradation products. A quadrupole mass spectrometer (MS) for the characterization of the compounds and a flame ionization detector (FID) for the quantitation were used.

2. Experimental

2.1. Chemicals and reagents

The following chemicals and reagents were used: sorbic acid (99%, EGA-Chemie, Germany), acetaldehyde (99.5%, Acros organics, Geel, Belgium), malonaldehyde bis (dimethyl acetal) (>99%), α-angelicalactone (98%), acrolein (99%), crotonaldehyde (99%, Acros organics, Geel, Belgium), acetone (99.5%, BDH, Poole, England), 2-methylfuran (>99%, Merck, Darmstadt, Germany), 2-acetyl-5-methylfuran (98%, Aldrich-Chemie, Deisenhofen, Germany), acetic acid (99–100%, Chem-lab, Belgium), 2-propanol (99.8%, Riedel-de Haën, Seelze, Germany), methanol (99.9%), ethanol (99.9%, Fischer scientific, Loughborough, England) and distilled water.

2.2. Pharmaceutical preparations

Different batches of cold cream (10 batches), cetomacrogol cream (14 batches) and vaseline (6 batches) were obtained from Pannoc Chemie, Olen, Belgium. They were from different manufacturing dates aging from 0 to 36 months. Blank preparations containing no sorbic acid were also available. These creams are used as base creams for pharmaceutical preparations.

2.3. Analytical instrumentation and parameters

2.3.1. HS-GC–MS

The gas chromatograph used was an Auto system XLGC (Perkin-Elmer, Foster city, CA, USA) with an autosampler for direct injection. The headspace autosampler used was a Turbomatrix HS 40 XL (Perkin-Elmer). The mass spectrometer was a Turbomass mass spectrometer (Perkin-Elmer) using electron ionization with a quadrupole mass analyser and a photomultiplier detector. The analytical column used was an AT-Aquawax-DA (Alltech, Dunkirk, NY, USA) with nitroterephthalic acid treated bonded polyethylene glycol as a stationary phase. For the experiments with direct injection, a narrow bore column (30 m × 0.25 mm) with a stationary phase film thickness of 0.25 μm was used. For the headspace experiments, a wide bore column (30 m × 0.53 mm) with a stationary phase film thickness of 0.5 μm was used.

The GC oven temperature was programmed with an initial temperature of 45 °C, isothermal for 8 min, then increasing with 16 °C/min to reach 230 °C and holding it for 10 min. The injection port temperature was maintained at 230 °C. The carrier gas used was helium 5.6 (Messer, Belgium). For the experiments with direct and HS injections, GC flow rates used were 1.0 and 4.0 ml/min, respectively. The MS parameters used were: 230 °C source temperature, 230 °C interface temperature, 70 eV ion energy, 0.5 s scan time and 0.1 s inter scan delay. The flow rate from GC to MS was maintained at 1.0 ml/min for both direct and HS injections. For the investigation in pharmaceutical preparations, the HS parameters maintained were: 90 °C thermostatting temperature for 15 min, 100 °C needle temperature, 120 °C transferline temperature, 180 kPa carrier gas pressure,

0.5 min vial pressurization time, 0.16 min injection time and 5 ml split flow programming.

2.3.2. HS-GC–FID

The gas chromatograph used was a Delsi 200 (Delsi-Nermag, Argenteuil, France) with FID. The headspace autosampler was a Dani 8650 (DANI, Milan, Italy). The analytical column used was the same wide bore column as used for HS-GC–MS.

The GC oven temperature program was adopted from the HS-GC–MS method. The carrier gas used was helium 5.6 at a flow rate of 4.0 ml/min. The FID temperature was maintained at 250 °C. The HS parameters were: 90 °C thermostatting temperature for 15 min, 120 °C manifold temperature, 150 °C transferline temperature, 0.5 min vial pressurization time, 1 ml injection volume and 20 ml GC injection split flow.

Twenty millilitre headspace vials and aluminum caps were obtained from Filter Service, Antwerp, Belgium.

2.4. Standards and sample preparations

Four oversaturated solutions were prepared: oversaturated solution I: 5 g of sorbic acid in 250 ml of water, oversaturated solution II: 5 g of sorbic acid in 250 ml of acetic acid, oversaturated solution III: as for I but kept at 60 °C and oversaturated solution IV: as for II but kept at 60 °C.

Standard solution I: 50 mg each of acetaldehyde; acrolein; crotonaldehyde; α-angelicalactone; malonaldehyde; 2-acetyl, 5-methylfuran and sorbic acid were dissolved in a mixture (50:50, v/v) of acetic acid and water.

Standard solution II: 125 mg each of acetaldehyde; acetone: acrolein; 2-methylfuran; crotonaldehyde; alfa-angelica lactone and 2-acetyl, 5-methylfuran were dissolved in 5 ml of DMSO and then diluted to 100 ml with water. From this solution, serial dilutions were made to obtain different concentration levels: 0.1, 0.5, 1, 5, 10, 30, 50, 100, 250, 500, 750 and 1000 μg/ml.

Standard solution III: a stock solution was made by dissolving acetaldehyde; acetone; 2-methylfuran and crotonaldehyde in 5 ml of DMSO and diluting to 100 ml with water. DMSO was used as a bridging solvent to dissolve the poorly aqueous soluble compounds. This solution was used to prepare further 5 dilutions resulting in concentrations of 125, 250, 375, 500 and 625 μg/ml for acetaldehyde; 2, 4, 6, 8 and 10 μg/ml for acetone; 0.5, 1, 1.5, 2 and 2.5 μg/ml for 2-methylfuran and 5, 10, 15, 20 and 25 μg/ml for crotonaldehyde.

Standard solutions IV: six different stock solutions corresponding to 0.05, 0.10, 0.15, 0.2, 1.0 and 2.0% of sorbic acid in water were prepared.

For the investigation of sorbic acid degradation in aqueous and acetic acid solutions the following samples were prepared:

For direct injection, 1.5 ml of each of the oversaturated solutions I–IV was filtered through 0.45 μm filters, placed in a 2 ml vial and 1.0 μl was injected. For headspace sampling, 5.0 ml of the oversaturated solution was placed in a 20 ml headspace vial and sealed.

Sample preparation for linearity, repeatability and detection limits:

The 5.0 ml of the respective concentration level from standard solution II was brought in a HS vial and sealed.

Sample preparation for estimation of degradation products in pharmaceuticals:

The 4.0 g of the preparation was put in a 20 ml headspace vial using a disposable plastic syringe, 1.0 ml of water was added and the vial was sealed. For the calibration, 4.0 g of sample and 1.0 ml of each concentration level of standard solution III was added. The sealed vials were vortexed for 0.5 min before placing them in the sampler.

3. Results and discussion

3.1. Investigation of sorbic acid degradation using direct injection

The 1.0 μl of standard solution I with expected degradation products of sorbic acid were injected on the GC–MS using direct injection. The parameters were optimized to attain a good separation and freshly prepared oversaturated solutions I–IV were injected. These solutions were investigated for any possible degradation peak during a period of 3 months. The MS was tuned towards low masses and the scanning parameters were optimized in a way to have enough data points (more than six) per peak to investigate its purity. Using the optimized GC–MS parameters, the AT-Aquawax-DA column showed a good separation between sorbic acid and its known degradation products (Fig. 1). The column showed low bleed at high temperatures and was found to be stable on long-term basis towards aqueous injections.

All freshly prepared oversaturated solutions I–IV gave clear chromatograms showing a sorbic acid peak together with trace amounts of acetaldehyde. This acetaldehyde could be either already present as an impurity in sorbic acid or it was produced by sorbic acid degradation in the GC injection port (230 °C). It was found that with increase in storage times, the degradation products of sorbic acid also increased in number and concentration. The degradation was found to be more pronounced in acidic than in aqueous media. Solutions at 60 °C showed more degradation than the ones at room temperature. The major degradation products, found in all solutions tested, were identified

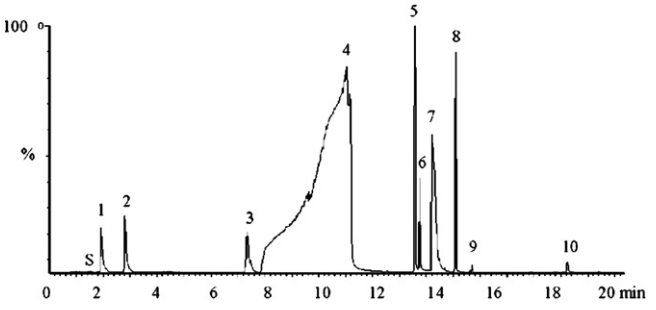

Fig. 1. Chromatogram obtained with standard solution I using HS-GC–MS parameters, described under experimental chapter. S: system peak, 1: acetaldehyde, 2: acrolein, 3: crotonaldehyde, 4: water, 5: α-angelicalactone, 6: acetic acid, 7: malonaldehyde, 8: 2-acetyl, 5-methylfuran, 9: angelicalactone related peak and 10: sorbic acid.

as acetaldehyde, acetone, 2-methylfuran and crotonaldehyde by their mass spectra. The identity of these compounds was confirmed by comparing the retention times with the retention times of the respective reference compound. 2-Methylfuran has not been reported before as a degradation product of sorbic acid.

Different extraction procedures using methylene chloride, chloroform and ethyl acetate were tried to extract the maximum concentration of degradation products from the oversaturated solutions. However, the extraction did not give any additional information. Further, it was observed that the extraction solvents were occupying most of the chromatogram and coeluted with peaks of interest. This might be problematic in the detection of new degradation products at low concentration or trace levels. Hence, sample preparation without extraction was preferred in further stages.

3.2. Investigation of sorbic acid degradation using HS sampling

Using the GC–MS method and standard solution I, different HS parameters such as thermostatting temperature, thermostatting time, needle temperature, transferline temperature and different pressures to pressurize the vial were investigated to allow repeatably a maximum concentration of degradation products into the headspace of the vial and later into the GC injection port with minimal carryover. As all the solutions and pharmaceutical preparations that were investigated contain water, thermostatting temperatures of more than 90 °C were not investigated. At such temperatures, the presence of water in the HS vial would cause overpressurization and also decomposition of the sample. In the range (50–90 °C) investigated, the HS concentration of volatiles increased with increasing thermostatting times. A thermostatting temperature of 90 °C was selected to achieve HS equilibrium in a short time since long exposure times to elevated temperatures could induce sorbic acid degradation. Among the different thermostatting times investigated (5, 10, 15, 30 and 45 min), the equilibrium between the liquid/sample and headspace was achieved within 15 min for all the compounds of interest. Different injection times, i.e. injection volumes were also examined to allow entering a maximum concentration of analyte from the headspace into the column without compromising the peak shape. An injection time of 0.16 min was selected. Using the optimized parameters, the standing oversaturated solutions I–IV were investigated. All the degradation peaks that were identified by direct injection were also found by HS sampling. In contrast to the direct injection, the degradation peaks were more intense (see an example in Fig. 2), but no new degradation peaks were detected. The sorbic acid peak was not found by HS sampling in aqueous solutions of sorbic acid (oversaturated solutions I and III) in contradiction with the oversaturated solutions II and IV, where acetic acid is present. So, undissociated sorbic acid is able to reach the headspace of the vial at low thermostatting temperatures (such as 80 °C).

Forced degradation was investigated also by adapting the thermostatting program to 120 °C for 30 min using oversaturated solutions I and II. This induced a series of sorbic acid degradation products. Together with the sorbic acid peak, a total of 12 peaks

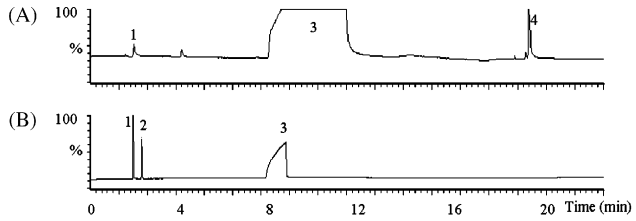

Fig. 2. Chromatograms of stored oversaturated solution I using two injection modes: (A) direct injection, (B) sHS injection. 1: acetaldehyde, 2: acetone, 3: water and 4: sorbic acid (unlabelled peaks are related to the blank matrix).

corresponding to degradation products were found (Fig. 3). Seven peaks, acetaldehyde; acetone; 2-methylfuran; toluene; crotonaldehyde; α-angelicalactone and 2,5-dimethylfuran were identified by their mass spectra and their identity was confirmed by comparing the retention times with those of their reference compound. Three peaks, cyclopropyl ethanone; pentenal and 2-methyl, 5-vinylfuran were identified by their mass spectra comparing to the reference spectra from National Institute of Standards and Technology (NIST) library. The mass spectra of the other two peaks (labeled as unknown in Fig. 3) were difficult to interpret. Structures of degradation products are listed in Fig. 4. Newly identified degradation products are 2-methylfuran; 2-methyl, 5-vinylfuran; 2,5-dimethylfuran and toluene. β-Carboxy acrolein, which was claimed to be one of the major degradation products of sorbic acid in literature was not found in either direct injection or HS investigation.

3.3. Headspace method validation

Sorbic acid is unstable and degrades at high temperatures. Since the headspace sampling involves heating the sample for a certain time, it must be verified whether the degradation perceived does not originate from the applied analytical method.

3.3.1. Effect of thermostatting temperature and time on the degradation of sorbic acid

Freshly prepared standard solutions IV were investigated on HS-GC–MS using a total sample volume of 5.0 ml in the HS vial. The investigated parameters were: thermostatting temperatures

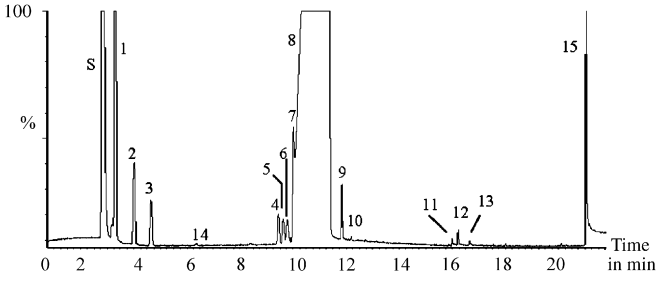

Fig. 3. Total ion chromatogram obtained with oversaturated solution I with elevated thermostatting program (120 °C for 30 min). S: system peak, 1: acetaldehyde, 2: acetone, 3: 2-methylfuran, 4: toluene, 5: crotonaldehyde, 6: cyclopropyl ethanone*, 7: pentenal*, 8: water, 9: unknown, 10: 2-vinyl-5-methylfuran*, 11: acetic acid, 12: unknown, 13: alfa-angelicalactone, 14: 2,5-dimethylfuran and 15: sorbic acid (* peaks identified only based on their mass spectra).

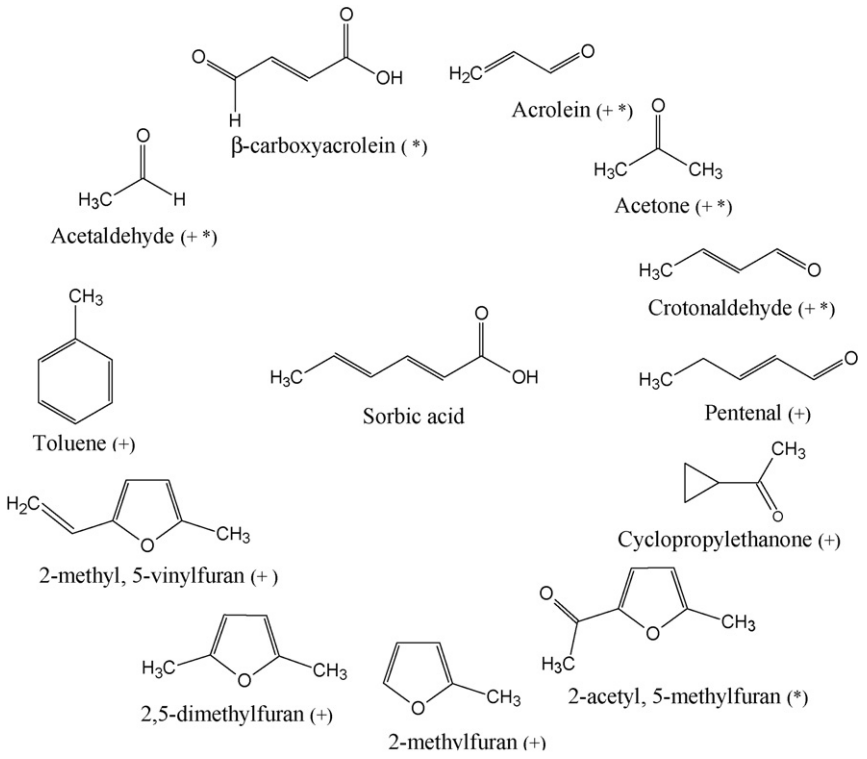

Fig. 4. Sorbic acid degradation products found (+) and/or reported in literature (*).

(80 and 90 °C) and thermostatting times (15, 30 and 45 min). At sorbic acid concentrations of 1.0 and 2.0%, in all the situations investigated, acetaldehyde; acetone; 2-methylfuran; toluene and crotonaldehyde were found. The HS equilibrium for these degradation products with water as a diluent at 80 and 90 °C could be achieved in less than 15 min. However, in this situation, the peak areas increased with the increase in the thermostatting time. Hence, it is a system-induced degradation. At sorbic acid concentrations less than 0.2%, with thermostatting times 30 and 45 min, only acetaldehyde (<2 μg) and 2-methylfuran (<0.1 μg) were found. At a thermostatting time of 15 min at both temperatures, only acetaldehyde (<1 μg) was found.

Most of the formulations using sorbic acid contain 0.1–0.15%. As the system-induced degradation at such concentrations was found to be very limited (around the detection limits of the system), a thermostatting program of 90 °C for 15 min was used for further investigation.

3.3.2. Repeatability, linearity, accuracy and detection limits

This quantitative part was performed on HS-GC–FID. Using standard solution II, the linearity was examined in the concentration range from 0.1 to 1000 μg/vial. Each concentration was injected in triplicate. All the compounds investigated showed good linearity with R^2 values more than 0.999. For the repeatability and recovery, concentration levels of 1, 5, 50 and 125 μg/vial were injected six times. At concentrations of 1 and 5 μg/vial the R.S.D. values were between 0.5–4% and for 50 and 125 μg/vial, 0.5–2%. The standard solutions were further diluted and injected to examine the detection limits. The validation data are presented in Table 1.

3.4. Sorbic acid content in investigated formulations

All the creams investigated contained 0.15% of sorbic acid on their date of manufacture. At that time the content was determined in our laboratory using a LC-UV method [29]. Some of the formulations were reanalysed for the sorbic acid content before investigating the degradation products. It was observed that 40 days after the date of manufacture, creams showed around 3% reduction from the initial content of sorbic acid. Three years after the date of manufacture all formulations showed from 93 to 97% reduction in sorbic acid content.

3.5. Investigation of degradation in pharmaceutical preparations

Different sample amounts were investigated, ranging from 1.0 to 6.0 g of sample in a vial. An amount of 4.0 g of sample in a vial yielded a maximum of degradation products to the headspace with the thermostatting parameters used in this study. Sample amounts above 4.0 g per vial did not show any further considerable increase in peak areas. All the samples investigated showed sorbic acid degradation products together with several blank peaks in the chromatograms. The blank peaks were discriminated after injecting the respective blank preparation containing no sorbic acid. No interference from the blank peaks was noticed in the sample chromatograms. The peaks not present in the blank were investigated using MS. The peak purity was confirmed by investigating the mass spectra at all the data points of each peak. All the batches of cold cream, cetomacrogol cream and vaseline showed mainly 4 degradation products

Table 1
Validation data of potential sorbic acid degradation products in water using HS-GC–FID (*R*: correlation coefficient, R.S.D.: relative standard deviation and s/n: signal to noise ratio)

	Concentration range (μg/vial)	*R* (>)	Linearity equation	Precision (R.S.D.) at 5 μg/vial (%)	Detection limits (s/n >3) in ng/vial	Average recovery (%)
Acetaldehyde	0.1–1000	0.999	$Y = 599\,X + 581$	3.2	15	98
Acetone	0.1–1000	0.999	$Y = 661\,X + 515$	2.6	80	101
Acrolein	0.1–1000	0.999	$Y = 531\,X - 2179$	4.0	100	96
2-Methylfuran	0.1–1000	0.999	$Y = 2589\,X + 875$	0.5	0.5	107
Crotonaldehyde	0.5–1000	0.999	$Y = 638\,X - 1748$	3.2	120	95
α-Angelicalactone	0.5–1000	0.999	$Y = 155\,X - 172$	3.8	500	98
2-Acetyl, 5-methylfuran	0.5–1000	0.999	$Y = 235\,X - 905$	3.6	300	95

of sorbic acid: acetaldehyde, acetone, 2-methylfuran and crotonaldehyde (Fig. 5). These results showed that sorbic acid is unstable in the preparations investigated, even shortly after manufacturing. The fact that the preparation of the creams involves heating would explain the presence of degradation products in the freshly prepared formulations. Acetaldehyde was found to be the major degradation product of sorbic acid in the preparations that were investigated. It was observed that the degradation is more pronounced in cetomacrogol creams followed by cold creams and vaseline. This observation demonstrated again that sorbic acid degrades more when more water is present.

3.6. Estimation of the degradation products

To add the reference compounds to the cream in the HS vial for the calibration, a certain amount of liquid had to be added. Using 4.0 g of cream different liquid volumes such as 1.0, 2.0 and 3.0 ml of water were investigated and there was no considerable difference of peak areas obtained with different liquid volumes. The combination of 4.0 g of cream with 1.0 ml of water was selected. The sample matrix effect on the headspace concentration of the degradation products in comparison to that of water as matrix was investigated by injecting 1.0 ml of standard solution II together with 4.0 g of cetomacrogol cream without sorbic acid in one vial and with 4.0 ml of water in the other vial. Cream as a sample matrix showed a little increase in sensitivity for acetaldehyde and acetone and a little decrease in sensitivity for crotonaldehyde. A drastic decrease in sensitivity was observed for 2-methylfuran followed by α-angelicalactone and acrolein (Fig. 6). This can be attributed to the fact that the headspace concentration of volatiles in a certain thermostatting program depends on the nature of the sample matrix. May be changing the thermostatting program would help to improve the headspace concentration for the ones with reduced sensitivity. However, in this investigation higher thermostatting temperatures and longer thermostatting times were ruled out due to the possibility of system-induced degradation of sorbic acid (see method validation).

Initially, quantification was tried on the HS-GC–MS using selected ion recording of the compound of interest. However, due to instability of the MS signal (continuous decay or gain of signal) final quantification was done using the HS-GC–FID. In total 10 samples of cold cream, 14 samples of cetomacrogol cream and 6 samples of vaseline were analysed for the identified sorbic acid degradation products. The analysis was performed using the standard addition method. The calibration was plotted using five different concentrations of the degradation products (dilutions from standard solution III). The calibration was found to be linear with R^2 values of more than 0.999. The results are shown in Table 2. Though the total amount of degradation products found could not explain for 100% the amount of sorbic acid degraded, acetaldehyde was found to be present in amounts related to the amount of sorbic acid degraded.

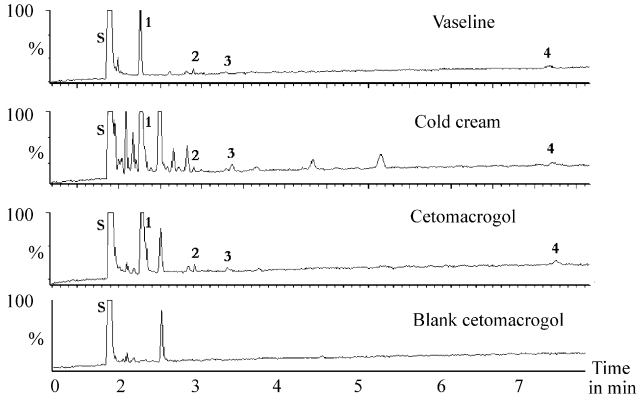

Fig. 5. Chromatograms of different pharmaceutical creams injected using HS-GC–MS parameters. S: system peak, 1: acetaldehyde, 2: acetone, 3: 2-methylfuran and 4: crotonaldehyde (unlabelled peaks are related to the blank matrix).

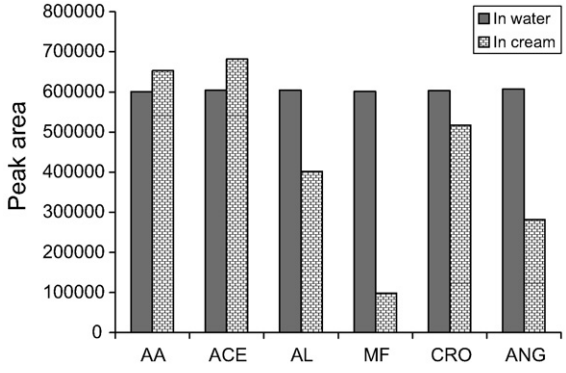

Fig. 6. Influence of the sample matrix on the headspace concentration of the analyte (in comparison with water as sample matrix). AA: acetaldehyde, ACE: acetone, AL: acrolein, MF: 2-methylfuran, CRO: crotonaldehyde and ANG: angelicalactone.

Table 2
Content of sorbic acid degradation products in pharmaceutical preparations at different age (0–36 months)

Sample	Concentration range observed (ppm)			
	Acetaldehyde (LD_{50}: 661)	Acetone (LD_{50}: 9750)	2-Methylfuran (LD_{50}: 167)	Crotonaldehyde (LD_{50}: 206)
Cold cream	5–300	0–10	0–1	0–10
Cetomacrogol cream	5–250	0–8	0–1	0–10
Vaseline	5–250	0–8	0–1	0–10

LD_{50}, lethal dose 50% in mg/kg determined on rats.

Table 3
Recovery from blank matrices

	Amount added in μg/vial	Amount recovered (%)
Acetaldehyde	62.5	96
	125	94
Acetone	5.0	100
	10.0	99
2-Methylfuran	5.0	99
	10.0	101
Crotonaldehyde	5.0	98
	10.0	98

3.6.1. Recovery studies

Blank matrices were spiked at two different concentration levels of each degradation product from standard solution III. Analysis was done using the standard addition method. The three matrices, cold cream, cetomacrogol cream and vaseline showed similar recoveries for all the compounds investigated. The recoveries were found to be within acceptable limits (Table 3).

4. Conclusions

Sorbic acid degrades in both aqueous and acidic media, even at room temperature. Acetaldehyde; acetone; 2-methylfuran; toluene; crotonaldehyde; 2-methyl, 5-vinylfuran; alfa-angelicalactone and 2,5-dimethylfuran were identified as sorbic acid degradation products. Two other peaks corresponding to sorbic acid degradation were also found, but were not yet identified. If the sample preparation allows direct injection to GC, the GC–MS/FID method can be used for simultaneous analysis of sorbic acid and its degradation products. Otherwise, the optimized and validated HS-GC–MS/FID method developed allows to identify and estimate the sorbic acid degradation products. As the samples used in this study could be uniformly distributed in water, the standard solutions were made using water as diluent. In the cases where the samples cannot be uniformly distributed in water, headspace compatible organic solvents (with a high boiling point and containing no volatile impurities) can be used for standard solutions and sample preparation. All the batches of cold cream, cetomacrogol and vaseline investigated showed four degradation products of sorbic acid: acetaldehyde, acetone, 2-methylfuran and crotonaldehyde. Given the quantities that can be expected during shelf life of the formulation and their toxicity limits (Table 2), it can be concluded that no real health risks are to be expected.

Acknowledgements

E. Adams is a post-doctoral fellow of the Fund for Scientific Research—Flanders (Belgium). Samples and blank matrices were gifted by Pannoc Chemie.

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