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Residual solvents in biodegradable microparticles: determination by a dynamic headspace gas chromatographic method

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Abstract

A sensitive and reliable method to determine the content of residual solvents in microparticles was developed. The microparticles were hydrolyzed in an aqueous solution of sodium hydroxide. The amount of residual solvent in this obtained polymer solution was then analyzed. Using a dynamic headspace gas chromatographic method the residual organic solvents were extracted, analyzed and evaluated with an internal standard. The applied method was optimized for methylene chloride with regard to precision, linearity, purge and trap conditions and stability of the internal standard.

Keywords: Dynamic headspace gas chromatography; Purge and trap gas chromatography; Organic volatile impurities; Microparticles; Residual solvent; Methylene chloride

1. Introduction

The production of injectable biodegradable microparticles with most of the common production techniques (Thoma and Schlütermann, 1991) led to considerable amounts of residual solvent in the particles. They are often toxic and, consequently, regulations formulated by the authorities have to be taken into account (Residual Solvents, 1990; US Pharmacopeia, 1995). Thus, a sensitive and reliable measurement of residual solvents in microparticles is needed. Gas chromatography (GC) is the method of first choice, but the sensitivity of direct injection techniques is poor. This is due to insufficient preparation of solid matrices, such as microparticles. Consequently, the headspace technique is favourable. Different authors worked with indirect headspace gas chromatographic devices (Hachenberg and Schmidt, 1977; Kolb, 1980; Ioffe and Vitenberg, 1984). Bicchi and Bertolino (1981) published a static headspace

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method for the determination of residual solvents in seven different drugs in the field of pharmacy. Guimbard et al. (1987) developed and optimized a general static headspace method for the determination of residual solvents in pharmaceutical products. Herlitz et al. (1987, 1988) developed a static headspace method for the analysis of pharmaceutical substances and excipients and investigated the residual solvent of packaging materials. Penton (1992) showed the suitability of static headspace GC to analyze residual solvents in pharmaceutical preparations performed with the solvents as laid down by the USP. However, a possible disadvantage of the static headspace is the dependence of the analytical results on the sample matrix (Hachenberg, 1988). Furthermore, it can lead to false results if more than one solvent is present in the sample. Additionally, the concentration in the vapour phase does not only depend on the partial pressure but also on the solubility of the substance in the solvent employed to dissolve the sample (Miller, 1990). Thus, one method the US Pharmacopeial Convention (1988) stipulates for investigation of residual solvents is dynamic headspace GC based on the US EPA Test Method 601 (1982). Wampler et al. (1985) investigated the residual solvent in aspirin tablets and other pharmaceuticals using dynamic headspace GC. Cyr et al. (1992) determined the residual solvent in captopril raw material and tablets with dynamic headspace GC. Thomas and Ramstad (1992) developed and validated a dynamic headspace GC method for the determination of vinyl chloride monomer in stored solutions of cefmetazole sodium in PVC bags.

In this study, a dynamic headspace GC method to analyze the amount of residual volatile organic solvents in microparticles was developed. The most important process parameters for this analytical method were determined and optimized.

2. Materials and apparatus

The employed organic solvents were methylene chloride for residue analysis (Merck, Darmstadt, Germany) and isopropyl chloride for analysis (Merck-Schuchardt, Munich, Germany). The solutions were prepared with sodium hydroxide for analysis (Merck, Darmstadt, Germany) and with double-distilled water. Resomer L-206 (Boehringer, Germany) was taken as biodegradable polymer. The sorbent material was Tenax TA, 20–35 mesh (Chrompack, Frankfurt, Germany).

The headspace apparatus consisted of the stripping device AMA LS-MA and the headspace unit AMA KA-D (AMA systems, Hilden, Germany), connected with a gas chromatograph from Hewlett Packard (HP 5880, USA). Headspace vials (N18-5) were used (Macherey and Nagel, Düren, Germany).

3. Experimental

3.1. Principle of the method

A schematic diagram of the dynamic headspace GC apparatus used is given in Fig. 1. The method can be described in two steps:

(a) The purge and trap procedure (Fig. 1a) is an extraction process of a liquid phase with an inert gas flow, followed by an enrichment of the extracted volatiles on a connected sorbent tube. This enrichment is due to an adsorptive process and allows the determination of trace amounts of organic volatiles.

The headspace-vial containing the aqueous solution of sodium hydroxide, the hydrolyzed microparticles, the internal standard and the residual solvent is positioned and incubated in the stripping device. A defined volume of nitrogen as inert gas is bubbling through the solution. The gas flow passes a sampling tube filled with a solid sorbent. In this tube the volatiles are trapped and enriched by adsorption.

(b) The second step (Fig. 1b) deals with the thermal desorption of the sampling tube after connection with the desorption unit of the gas chromatograph. During this step the desorbed compounds are again preconcentrated in a cold trap cooled with liquid nitrogen in front of the capillary column. The fast heating up of the cold trap accompanied by the start of the GC system results in a very small starting peak width which

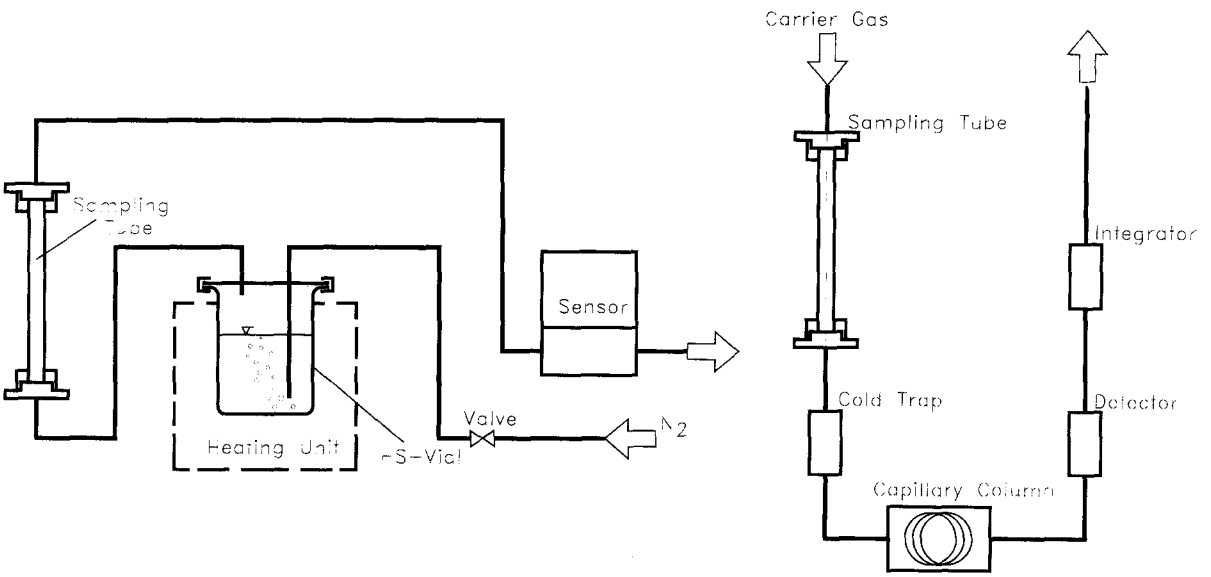

Fig. 1. (a) and (b): Diagram of the dynamic headspace gas chromatographic method.

allows optimum utilization of the separation efficiency of the capillary column.

3.2. Calculations

The calculation of the residual solvent of the microparticles was performed using the following equation.

$$C_{CH_2Cl_2} = \frac{A_{CH_2Cl_2} \cdot W_{ST} \cdot 10^6}{A_{ST} \cdot RF \cdot W_{Mp}} \quad (1)$$

$C_{CH_2Cl_2}$: Amount of residual methylene chloride (ppm)
$A_{CH_2Cl_2}$: Peak area of methylene chloride
W_{ST}: Weight of the internal standard (μg)
A_{ST}: Peak area of the internal standard
RF: Response factor between methylene chloride and internal standard
W_{Mp}: Weight of analyzed microparticles (μg)

The response factor was calculated by analyzing solutions containing known concentrations of methylene chloride and internal standard:

$$RF = \frac{A_{CH_2Cl_2} \cdot W_{ST}}{A_{ST} \cdot W_{CH_2Cl_2}} \quad (2)$$

$W_{CH_2Cl_2}$: Weight of methylene chloride [μg]

Employing isopropyl chloride as internal standard, the average value of the response factor was 0.45. This factor was proved on each day with standard solutions of methylene chloride and internal standard. A deviation of $\pm$ 10% was tolerated.

3.3. Sample preparation

The microspheres (20–50 mg) were filled in headspace vials with volumes of approximately 5 ml and a solution of 1 ml 1-N sodium hydroxide containing isopropyl chloride (4 μg/ml) was added. The vial was quickly closed with a screw cap and a rubber septum with a PTFE surface The suspension was shaken for 24 h at room temperature. The hydrolysis of the microparticles resulted in a clear solution. After incubation of the vial the purge and trap procedure was started.

3.4. Optimization of the method

The investigations were carried out with solutions of sodium hydroxide, polymer, methylene

chloride and isopropyl chloride. The following aspects were investigated.

The precision of the method was determined with solutions of different concentrations of methylene chloride in a range of 10–11.78 μg/ml. Isopropyl chloride was added as internal standard in a concentration of approximately 4 μg/ml to some solutions. The stripping temperature was 30°C and the stripping volume of nitrogen was 200 ml.

The linearity of calibration was investigated using solutions with five different concentrations of methylene chloride. These concentrations were adequate to a range of 0.98–10.9 μg methylene chloride. The solutions were stripped with a nitrogen volume of 200 ml at a temperature of 30°C. A calibration curve was calculated and a regression analysis with and without internal standard was carried out. The 95% confidence interval for the intercept of the y axes and the slope of the curve was calculated. Each concentration of methylene chloride was analyzed a minimum of two times.

The influence of different purge volumes (including breakthrough volume of the Tenax column) was investigated by stripping a solution of methylene chloride (12.9 μg) with different purge volumes in the range 100–750 ml. The response factor of methylene chloride and isopropyl chloride was calculated for each purge volume.

The influence of the purge temperature was analyzed using a solution with a concentration of methylene chloride of 10.43 μg/ml. The solution was stripped with a volume of 200 ml at different incubation temperatures in the range 25–50°C. The temperature was varied in 5°C intervals and the incubation time was 30 min.

The stability of the internal standard and methylene chloride in the headspace vial was analyzed. 1 ml of a solution with 4.3 μg methylene chloride and 2.8 μg isopropyl chloride was filled in headspace vials and shaken at room temperature for 72 h. After different periods of time, samples were analyzed. The peak areas of methylene chloride and internal standard, as well as the response factor, were evaluated.

Before each day of analysis, the sorbent tubes as well as the cool trap were heated at 230°C for 15 min to remove trace amounts of residuals. The success of the removal was checked with a blank analysis. After each run of analysis, the cleaning procedure mentioned above was repeated.

4. Results and discussion

Microparticles as a solid matrix system require a dissolution step before an analysis of the total incorporated residual solvent is possible. Organic solvents such as DMF, dimethylacetamide or dioxane have a limited capacity to dissolve biodegradable polymers. Furthermore, the disadvantage of a marked solvent peak in the chromatogram occurs and can lead to false results (Thomas and Ramstad, 1992). In contrast to these drawbacks, the hydrolysis of the microparticles in aqueous alkaline solutions is suitable for most biodegradable polymers. Additionally, a solvent peak is absent. Extracted water vapour shows no peak in the chromatogram because it is not detected by the Flame Ionization Detector (FID). Nevertheless, it has been taken into account that water vapour is able to shift the retention times of the eluted volatiles in the chromatographic system (Thomas and Ramstad, 1992). In the present method, this occurred in the range of 10.5–12 min for methylene chloride and 12–13.5 min for isopropyl chloride.

Isopropyl chloride shows a similar polarity and retention time to methylene chloride as shown in the chromatogram illustrated in Fig. 2. Thus, it was a favourable internal standard in the presented study. The vapour pressure (695 hPa at 20°C) of isopropyl chloride is higher than the pressure of methylene chloride (453 hPa at 20°C). The added internal standard is therefore more sensitive than the analyzed solvent. This helps to recognize and avoid mistakes in the sample preparation or the analyses. The solubility of isopropyl chloride in aqueous solutions (3.1 g/l) is sufficient to prepare stock solutions of the internal standard. These are diluted to the applied concentration of approximately 4 μg/ml.

As demonstrated in Fig. 3, the analytical results were influenced by the stripping temperature. In the range 25–50°C, the peak areas of methylene

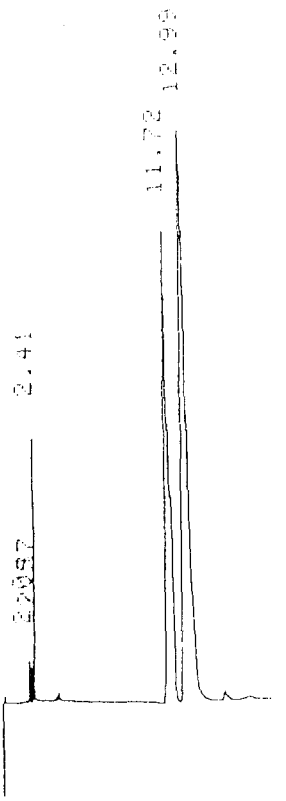

Fig. 2. Chromatogram of methylene chloride and isopropyl chloride.

chloride as well as the internal standard increased at lower temperatures. At temperatures of 35–50°C, there is only a slight increase in the peak areas. The fluctuation of the measurements could be the reason. It can be seen that the temperature

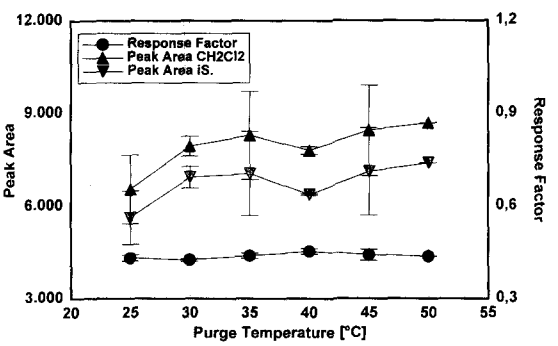

Fig. 3. Influence of the stripping temperature (mean ± S.D.).

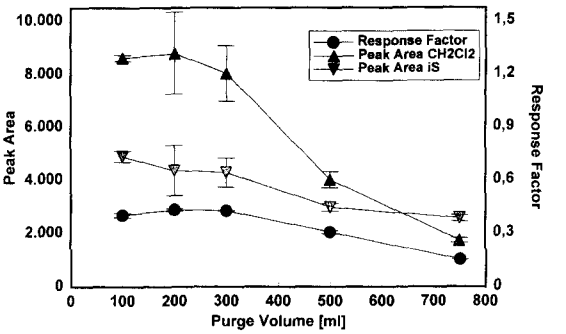

Fig. 4. Influence of the stripping volume (mean ± S.D.).

did not affect the response factor and the analytical results, but when working at temperatures above 35°C, an increasing amount of water vapour was extracted and later on trapped in the cool trap at −120°C. Freezing of the trap capillary led in a few runs to a breakdown of the carrier gas flow and the analysis had to be stopped. The same effect was reported by Driss and Boughuerra (1991). A temperature of 30°C was therefore chosen as standard purge temperature.

The peak areas of methylene chloride and isopropyl chloride in the test series of different purge volumina were unchanged up to a volume of 200 ml (Fig. 4). With a purge volume of 300 ml, the peak areas of the two volatiles began to decrease. The resulting response factor was constant up to a purge volume of 300 ml. The decrease of the peak areas with increasing purge volumes is due to the exceeding of the breakthrough volume of the sorbent column used.

The precision of the method was determined with solutions, including methylene chloride concentrations listed in Table 1 with and without an internal standard. A variation coefficient (RSD) of the peak area between 10.33 and 14.85% was observed when evaluating the data without an internal standard. The solutions containing internal standard showed an RSD of the peak area from 11.74 to 14.21%, but the RSD of the concentration of methylene chloride calculated with the internal standard is below 5%. Hence, the addition of an internal standard is favourable to get reproducible analytical results.

Table 1
Accuracy of the analytical method

Conc. CH_2Cl_2 (μg/ml)	No. of analyses	Mean peak area of CH_2Cl_2	RSD CH_2Cl_2 (%)	RSD CH_2Cl_2 calc. w. iS (%)
12.16	8	8957.8	14.85	—
10.44	11	7229.9	11.16	—
10.42	7	7324.2	10.33	—
3.9	7	2021	14.21	4.4
5.9	7	3205.1	13.09	4.3
4.8	9	2832.5	11.74	4.2

w. iS, with internal standard.

In Fig. 5, a plot of calculated concentrations of methylene chloride versus analyzed methylene chloride is shown. Evaluation with an internal standard resulted in better regression coefficients than evaluation of the peak areas of methylene chloride. The response factor between methylene chloride and internal standard did not change significantly over the investigated concentration range of methylene chloride. Calculation of regression of seven different calibration curves with internal standard resulted in an average regression coefficient of 0.997. The coefficient fits the proposal of the dynamic headspace method of the US Pharmacopeial Convention (1988). The y intercept is not significantly different from zero on a 95% confidence level and the slope of the curve does not significantly differ from one.

The stability of methylene chloride and isopropyl chloride in the aqueous medium of sodium hydroxide was analyzed over 72 h. Fig. 6 shows no decrease in the peak area of methylene chloride or isopropyl chloride and the response factor varied in a range of 4.3% RSD for all response factors. The response factor did not change during the observed course of time. This indicated no leakage of the headspace vials or any chemical interaction of the solvents during the hydrolyses of the microparticles.

The resulting experimental standard conditions for the determination of residual methylene chloride in microparticles are listed below:

- Stripping unit: AMA LS-MA
  Incubation time: 30 min; stripping temperature: 30°C; stripping volume: 200 ml; sorbent tube: Combirohr CBR 150 filled with Tenax TA.
- Desorption unit: AMA KA-D
  Desorption temperature: 200°C; desorption time: 5 min; temperature of the cold trap: 120°C (cooled with liquid nitrogen); rate of carrier gas flow during desorption: 10–15 ml/min; heating rate of the cold trap: within 1–1.5 min from −120 to +200°C; heating time of the cold trap: 5 min.

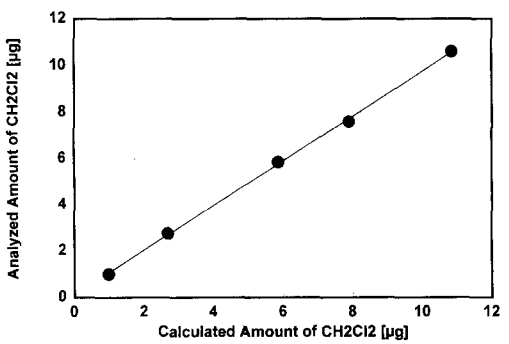

Fig. 5. Calibration curve of analyzed vs. calculated methylene chloride.

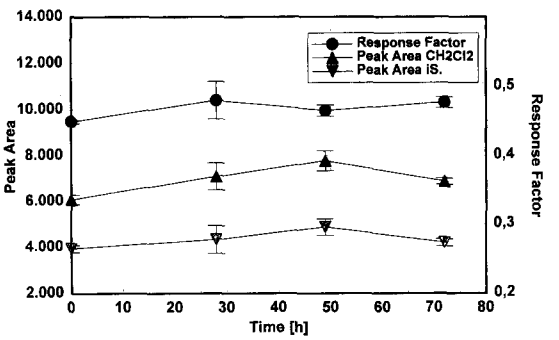

Fig. 6. Stability of methylene chloride and isopropyl chloride in 1 N sodium hydroxide (mean ± S.D.).

- GC analysis apparatus: Hewlett Packard 5880 Detector: FID; temperature of the detector; 250°C; carrier gas: helium; carrier gas pressure: 1 bar; carrier gas flow: 1.2 ml/min; column: Chrompack Capillary column (25 m), coated with Poraplot Q (10 μm film thickness); internal diameter 0.32 mm; oven temperature: 135°C for 17 min, 135–180°C with a rate of 10°C/min, 180°C for 10 min; make up gas: nitrogen (30 ml/min); air flow: 400 ml/min; hydrogen flow: 30 ml/min.

5. Conclusions

The developed analytical dynamic headspace GC method is appropriate to determine the residual solvent in biodegradable microparticles. Optimization of the method ensured that the proposals of the US Pharmacopeial Convention were fulfilled.

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