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Improving quantitative gas chromatography–electron ionization mass spectrometry results using a modified ion source: Demonstration for a pharmaceutical application

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ABSTRACT

Gas chromatography–mass spectrometry is a well established analytical technique. However, mass spectrometers with electron ionization sources may suffer from signal drifts, hereby negatively influencing quantitative performance. To demonstrate this phenomenon for a real application, a static headspace-gas chromatography method in combination with electron ionization-quadrupole mass spectrometry was optimized for the determination of residual dichloromethane in coronary stent coatings. Validating the method, the quantitative performance of an original stainless steel ion source was compared to that of a modified ion source. Ion source modification included the application of a gold coating on the repeller and exit plate. Several validation aspects such as limit of detection, limit of quantification, linearity and precision were evaluated using both ion sources. It was found that, as expected, the stainless steel ion source suffered from signal drift. As a consequence, non-linearity and high RSD values for repeated analyses were obtained. An additional experiment was performed to check whether an internal standard compound would lead to better results. It was found that the signal drift patterns of the analyte and internal standard were different, consequently leading to high RSD values for the response factor. With the modified ion source however, a more stable signal was observed resulting in acceptable linearity and precision. Moreover, it was also found that sensitivity improved compared to the stainless steel ion source. Finally, the optimized method with the modified ion source was applied to determine residual dichloromethane in the coating of coronary stents. The solvent was detected but found to be below the limit of quantification.

1. Introduction

Gas chromatography (GC) in combination with electron ionization mass spectrometry (EI-MS) is a well-recognized, established analytical technique [1–5]. The combination of the high separation efficiency of GC together with mass spectral information of (unknown) analytes makes this hyphenation enormously attractive for a wide range of analytical applications. Moreover, valuable time can be gained as identification and quantification of unknown compounds may be performed in the same run. Hence, such instrumentation can be found in diverse environments (e.g. QA/QC, research, process control) in different fields (e.g. pharmaceutical, biomedical, food, chemistry, etc.) [6–9].

Although benchtop EI-MS instruments are considered state-of-the-art nowadays, one should pay attention to the quantitative performance. In a previous study in our laboratory signal drifts were reported as a result of the stainless steel design of the EI source [10]. Hence, accurate quantification may not be possible. A practical and economic modification to the ion source reduced signal drifts remarkably and improved quantitative performance. Here, this improvement is demonstrated for the quantitative analysis of residual dichloromethane in coronary stent coatings. So far, there are no reports about residual solvent determination in stent coatings.

Pharmaceutical aspects of drug eluting stents have been discussed [11]. As stent coatings may act as carriers for controlled drug release, they are considered as pharmaceutical excipients and thus should meet quality requirements of pharmaceuticals. One of the guidelines issued by the International Conference on Harmonisation (ICH) is the identification and control of residual solvents (RS) [12,13]. RS are defined as volatile organic compounds found in bulk active pharmaceutical ingredients, excipients or finalized drug products. These solvents were used or produced during the manufacturing process of the substance. Appropriate solvent selection may enhance the yield or give the product its preferred physicochemical properties [14]. According to the ICH guideline,

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Table 1
Optimized sHS parameter settings.

Parameter	Setting
Thermostatting temperature	80 °C
Needle temperature	90 °C
Transferline temperature	120 °C
Equilibration time	30 min
Pressurization time	1.0 min
Injection time	0.08 min
Injection pressure	150 kPa

dichloromethane is a class 2 RS with a concentration limit of 600 ppm.

The aim of this work is to demonstrate the difference in quantitative performance using a standard and a modified ion source for the abovementioned application. To this purpose, a static headspace (sHS) GC–EI-MS method was developed and validated for the quantitative determination of residual dichloromethane in stent coatings. Values for linearity, precision and limit of detection/quantification (LOD/LOQ) obtained with the standard EI source were compared with those of the modified source.

Finally, the optimized method using the modified ion source was applied to real sample batches.

2. Experimental

2.1. Materials and reagents

HPLC grade dichloromethane was purchased from Acros Organics (Geel, Belgium). Dimethyl sulfoxide (DMSO) for gas chromatography was bought from Merck (Darmstadt, Germany). Sample stents with known amounts of coating, uncoated stents and solvent-free coating were received from Ziscoat (Heverlee, Belgium). Ultrapure water was produced in the laboratory with a Milli-Q® water purification system from Millipore (Molsheim, France). The 22 ml headspace vials and aluminium crimp caps were obtained from Filter Service (Eupen, Belgium).

2.2. Sample preparation

Three stents were directly placed into a headspace vial. To dissolve the coating (about 1.2 mg in total), 1.0 ml of DMSO was pipetted into the vial. Hereafter, 1.0 ml of water was added, reaching a total volume of 2.0 ml.

A reference stock solution of dichloromethane at a concentration of 1.0 μg ml^{-1} was prepared in a suitable dilution medium. The latter was made as follows: solvent-free coating was dissolved in DMSO to obtain a concentration of 1.2 mg ml^{-1}. Reference vials contained three uncoated stents, 1.0 ml of reference solution or an appropriate dilution and 1.0 ml of water. Blank vials consisted of 3 uncoated stents, 1.0 ml of DMSO and 1.0 ml of water.

2.3. Instrumentation

All instruments and software were from Perkin Elmer (Waltham, MA, USA). The headspace sampler was a Turbomatrix HS40 autosampler. The headspace parameter settings are listed in Table 1. The GC instrument was an Autosystem XL. The GC-column was an AT^{TM}-Aquawax (30 m × 0.53 mm × 0.5 μm) obtained from Grace (Deerfield, IL, USA). The GC temperature program was as follows: after an initial temperature of 50 °C held for 5 min, the oven was heated to 180 °C at 40 °C min^{-1}. The final temperature was held for 10 min. The injection port temperature was maintained at 140 °C. Helium 5.6 was used as carrier gas at a flow rate of 4.0 ml min^{-1}.

The MS was a turbomass EI-quadrupole instrument. The MS transferline and ion source temperatures were set at 180 °C and 250 °C, respectively. The electron energy used was 70 eV. For the detection of dichloromethane, single ion recording (SIR) at m/z 49 was applied. For optimal comparison between the investigated ion sources, both sources were cleaned before experiments were run. For cleaning the stainless steel ion source parts, they were initially polished with abrasive powder. Next, they were degreased by sonication in a methanol solution. Cleaning gold coated ion source parts was done by sonicating in a dilute ammonia solution first and afterwards in a dilute citric acid solution. When a freshly cleaned ion source was inserted, an automatic tuning procedure was run by the software. After the autotuning procedure, the repeller voltage was fixed at 1.0 V for better evaluation of ion source performance. Finally, the MS was calibrated before running the experiments. Turbomass software was used for data processing.

2.4. Ion source modification

A gold layer of 5 μm in thickness was electroplated over a protective nickel layer on the existing surface of a spare repeller and exit plate. Gold coating procedures were carried out at Britech Private Ltd. (Hyderabad, India). All other parameters remained identical when the modified ion source was deployed.

2.5. Validation

As headspace sampling is known as a repeatable injection technique and sample loss is not likely to occur in this work, external calibration was chosen as quantification method. Moreover, external calibration permits clear performance comparison between the stainless steel ion source and modified ion source. Unless mentioned otherwise, the sHS–GC–MS method was validated according to the guideline for validation of analytical methods of ICH [15].

2.5.1. Limit of detection and limit of quantification

To determine the limit of detection (LOD) and limit of quantification (LOQ), the reference stock solution was further diluted with dilution medium. One milliliter of dilute reference solution was added to a vial containing 1.0 ml of water. The amount of dichloromethane in the vial that resulted in a S/N ratio of 3 was taken as LOD, whereas for LOQ this was a S/N ratio of 10. The noise was calculated in a time interval of 30 s next to the dichloromethane peak. In case of blank interference, calibration curves were recorded in a small range around the estimated LOQ value. Then LOD and LOQ were calculated from regression data according to the formulas LOD = (3.3*σ)/S and LOQ = (10*σ)/S, where σ = standard error of intercept and S = slope of the curve.

2.5.2. Linearity

The minimum linearity range to be considered according to ICH is from LOQ to (at least) 120% of the limit value. For dichloromethane, the limit concentration is 600 ppm. As the total sample amount in the vial was about 1.2 mg, this corresponds to a limit amount of 720 ng in the vial. Hence, linearity was investigated in a range starting from LOQ to an amount of 1 μg in the vial. Over this range, five quantity levels were prepared by serial dilution of the reference stock solution with dilution medium. Each concentration step was analyzed in triplicate. Also here, all vials contained 1.0 ml of appropriate reference dilution and 1.0 ml of water.

2.5.3. Precision

Precision was evaluated by calculating the relative standard deviations (RSD) of the peak areas (n = 3) obtained at each concentration step of the linearity experiment.

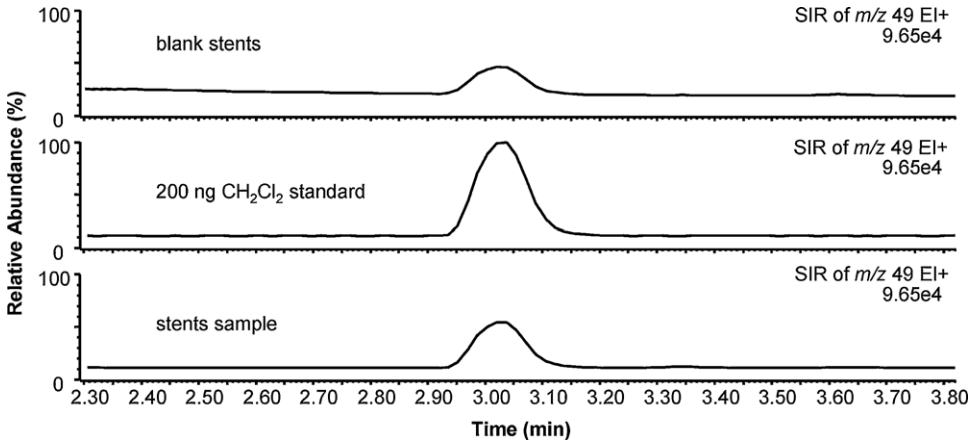


Fig. 1. Representative sample chromatogram (3 stents) together with a chromatogram of the lowest calibration standard and a blank chromatogram obtained from the analysis of three solvent-free stents.

2.6. Method application on real sample

The optimized sHS–GC–MS method was applied to real stent samples. Three stents, containing coating of the same batch, were directly placed in a vial and analyzed.

3. Results and discussion

3.1. Method optimization

The method was developed with the parameter settings mentioned in the Ph. Eur. method for the determination of RS as a starting point [9]. First, the GC run time was reduced from 58 min to 18.3 min by shortening the oven temperature program. Preliminary experiments revealed that residual dichloromethane concentrations in the stent coating were very low. Therefore, stents were directly placed into the vials avoiding any dilution of the sample. The coating was dissolved in the vial by adding 1.0 ml of DMSO. To further enhance sensitivity, 1.0 ml of water was added. The addition of water decreases the partition coefficient of dichloromethane, resulting in higher gas phase concentrations. The addition of water to organic dilution media to improve sensitivity was first described by Steichen [16].

Headspace settings were also optimized. As complete equilibration was achieved in less than 30 min, the equilibration time was decreased from 60 min to 30 min. The pressurization time was increased from 0.5 min to 1.0 min to obtain better precision. Needle and transferline temperatures were set to slightly higher temperatures to avoid condensation.

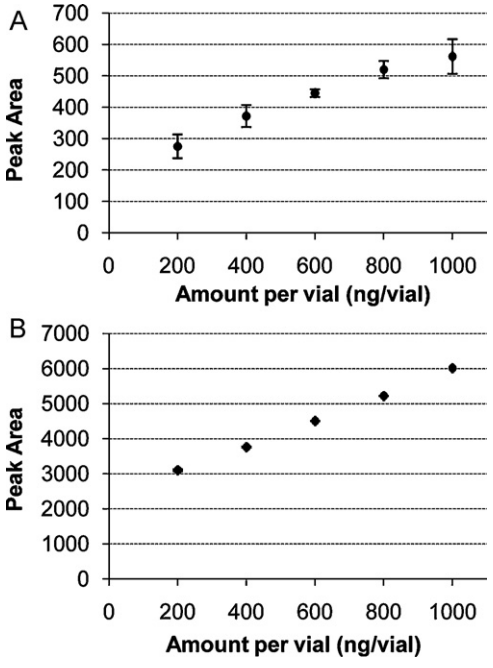


Fig. 2. Calibration points obtained with the stainless steel ion source (A) and with the modified ion source (B). The error bars represent the standard deviation of the mean peak areas.

3.2. Chromatography

The retention time of dichloromethane in the SIR (*m*/*z* 49) chromatograms was 3.0 min. As there was only 1 analyte of interest, selectivity was not an issue. Blank vials were analyzed to check for possible matrix interference. No interfering peaks were noticed. With the modified ion source however, blank interference with the same retention time of dichloromethane was observed and could not be avoided, despite complete system maintenance. As the blank response was found to be constant, it was decided to take it into account in all further experiments and calculations. SIR traces for a representative blank, calibration standard and stent sample are shown in Fig. 1.

3.3. Validation

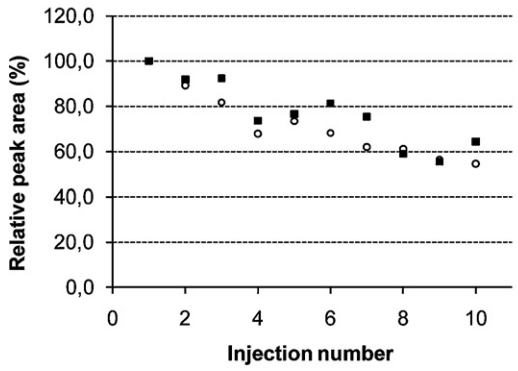
First, the optimized method was validated employing the original stainless steel ion source of the MS. Validation was then repeated using the modified ion source. Validation results using both ion sources were compared and are shown in Table 2.

3.3.1. LOD/LOQ

Using the original ion source, LOD and LOQ were taken as those concentrations giving a S/N ratio of 3 and 10, respectively. LOD and LOQ were found to be 60 ng and 200 ng of dichloromethane in the vial. When using the modified ion source, blank interference was observed, even after complete HS and injector maintenance. Hence it was decided to incorporate blank vials in all upcoming sequences and take the blank responses into account in all calculations. Since the S/N ratio cannot be used to determine LOD and LOQ, they were derived from the regression data obtained with the linearity experiment. LOD and LOQ were found to be 24 and 73 ng of dichloromethane in the vial, respectively. When this method of LOD/LOQ calculation was applied to the regression obtained with

Table 2
Comparison of the validation results obtained with the stainless steel (SS) and the modified ion source. The RSD values represent the lowest and highest value obtained over the different concentration levels analyzed for the linearity.

Ion source	LOD (ng/vial)	LOQ (ng/vial)	Range (ng/vial)	Linear equation	R^2	RSD (%) ($n = 3$)
SS	60	200	200–1000	$y = 0.40x + 219$	0.9831	2.8–13.7
Modified	24	73	200–1000	$y = 3.59x + 2373$	0.9993	0.1–2.8



Injection nr.	Response factor
1	3.68
2	3.57
3	3.25
4	3.39
5	3.53
6	3.09
7	3.02
8	3.80
9	3.74
10	3.12
Mean	**3.42**
Sd	0.29
RSD (%)	**8.4**

Fig. 3. On the left, graph showing the peak areas of dichloromethane (○) and chloroform (■) obtained from a series of 10 consecutive injections. On the right, the response factors (peak area dichloromethane/peak area chloroform) calculated for each injection.

the original source, LOD and LOQ were 190 ng/vial and 590 ng/vial, respectively. Consequently, because of worse regression, LOD and LOQ values were considerably higher than those obtained with S/N calculation.

3.3.2. Linearity

To assess linearity, a calibration curve was recorded ranging from 200 ng to 1000 ng of dichloromethane in the vial. Five equally distributed quantity levels were prepared and each analyzed in triplicate. Calibration curves are shown in Fig. 2. When using the original ion source, a non-linear relationship was obtained with $R^2 = 0.9831$. When fitting a second-order polynomial function, the R^2 value was 0.9988. The same range was also investigated with the modified ion source. Contrary to the original ion source and using identical sHS–GC–MS parameter settings, a linear relationship was obtained with $R^2 = 0.9993$.

3.3.3. Precision

Instrument repeatability was assessed by calculating the RSD ($n = 3$) of the peak areas obtained by replicate injections of each quantity level of the linearity experiment. Using the original ion source, RSD values ranged from 2.8 to 13.7%. With the modified ion source RSD values ranging from 0.1 to 2.8% were obtained. RSD of the blank response was 4.8%.

3.4. Using an internal standard?

An internal standard compound could be useful to neutralize the signal drift effect, provided the drift equally affects analyte and internal standard. This condition was tested with chloroform as internal standard. A series of 10 vials, containing dichloromethane and chloroform each at a fixed amount of 1 μg/vial, were injected. The peak areas of the compounds decreased continuously, as expected. The RSD values ($n = 10$) for dichloromethane and chloroform were 21% and 19%, respectively. A plot of the relative peak areas (with respect to the peak areas obtained with the first injection) is shown in Fig. 3. It can be seen that the progress of signal drift slightly differed. The response factors (Rf) were calculated by dividing the peak area of dichloromethane with that of chloroform. The mean Rf was 3.42 but with an RSD of 8.4% ($n = 10$). This high RSD value reflects the different influence on the response of both compounds, affecting quantitative accuracy. Consequently, proper internal standard selection is of great importance using electron ionization MS. In fact, only deuterated analogues should be used as internal standard.

3.5. Sample analysis

Three stents were analyzed together in 1 vial using the optimized method with the modified ion source. It was found that the residual dichloromethane concentration for the three stents together was below LOQ (73 ng/vial) but above LOD (24 ng/vial). Although the concentration was too low to quantify, it could only be detected using the modified ion source owing the better sensitivity. With the original ion source, no peak would be observed.

4. Conclusions

In this work the quantitative performance of an original, stainless steel electron ionization ion source was compared with an ion source containing a gold coated repeller and exit lens. Comparison was done by validating an optimized analysis method for the quantification of residual dichloromethane in triglyceride stent coatings. For easy interpretation, headspace sampling was used with external calibration as quantification method. Over an identical range, linearity and precision were evaluated and compared. It was found that, due to expected signal drift, a non-linear response was obtained with the original ion source. As a consequence, RSD values were higher than 10%. Using an internal standard with the stainless steel ion source may improve validation results. However, it was found that response factors also fluctuate, even with closely related compounds, possibly leading to inaccurate results. With the modified ion source however, a linear relationship was obtained over the same range with all RSD values lower than 3%. Moreover, LOD and LOQ concentrations were found to be lower using the modified ion source. Hence, not only signal drift improved, but also the sensitivity was enhanced using the modified ion source.

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