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Validated Static Headspace Gas Chromatographic Method for Determination of Formic Acid in Pharmaceutical Excipients

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Abstract : A static headspace gas chromatographic method (SHS-GC) was developed and validated for determination of formic acid after derivatizing it to ethyl formate using acidified ethanol. The developed method was simple and specific. It has acceptable accuracy and precision. The limits of detection and quantitation of formic acidwere127.15 and385.29 µg/L, respectively. This method was successfully used to determine formic acid impurity in pharmaceutical excipients.
Keywords : Formic acid, Pharmaceutical excipients, Chemical derivatization, Static headspace, Gas chromatography.

1. Introduction

Although considered pharmacologically non-active, pharmaceutical excipients have critical effects on drug product safety, efficacy and quality. The stability of many drug substances can be negatively influenced not only bydirect reaction with excipients, but also by reaction with their impurities. These impurities include formic acid which is classified as a reactive impurity in pharmaceutical excipients [1].

Formic acid may be found in many excipients especially in polyvinylpyrrolidone(PVP),polyethylene glycols(PEGs) and non-ionic surfactants. It can be mainly formed by oxidative degradation [2].

Formic acid is one of the low toxic class III solvents which are not specified or quantified in pharmaceuticals till it exceeds the limit of 0.5%.This limit is defined according to toxicological considerations only [3].However, lower levels of formic acid may cause instability of drug products as it can react with amino and/or hydroxyl groups in many pharmaceuticals. This leads to formation of the corresponding amides, as happened withvarencline, and/or esters degradation products. It can also modify formulation pH and form salts with basic drugs [4, 5]. Moreover, formic acid has accelerated the degradation of the experimental drug FK480 by optical isomerization from the desired S-enantiomer to undesired R-enantiomer [6].

Formic acid is volatile and highly reactive. Furthermore, it has little UV activity andlow detectors sensitivity. So, it is difficult to detect and determine formic acid readily. Many analytical methods have been developed to analysis formic acid such as colorimetric, spectrophotometric, CE, GC, HPLC and ion chromatographic methods. Lots of these methods employ chemical derivatization before formic acid determination [7-10]. Colorimetric and spectrophotometric methods have not sufficient specificity and/or sensitivity. Ion exchange chromatographic methods have in adequate specificity because of the susceptibility to interfering ions that may be originated from the used reagents, solvents, glassware or from the sample itself [11].Some HPLC and GC methods have not sufficient specificity and/or sensitivity. While others require multi-

steps and time-consuming sample preparation procedures. Many reported methods have been derivatized formic acid by alcohols before gas chromatographic determination [7, 10, 12-14].Barrio et al. reported a HS-GC-MS method for determination of formic acid in pharmaceutical excipients by converting it to ethyl formate using acidified ethanol. The sample preparation was very simple and rapid, and the chemical derivatization can be carried out under mild conditions. This method was very specific and sensitive. However, it requires MS detector and sophisticated instrumentation which are not available in most typical laboratories [7].

The aim of this research was to develop and validate a SHS-GC-FID method toquantify formic acid in pharmaceutical excipients, and to use it for screening the excipient samples obtained from different sources.

Experimental

1. Standards, reagents and chemicals

Ethyl formate standard(≥ 99.5%) was purchased from Fluka (Switzerland). Absolute ethanol and pure formic acid were purchased from Panreac (Spain). ACS grade *p*-toluene sulfonic acid monohydrate (≥ 98.5%) was purchased from Sigma-Aldrich (Japan). All excipients samples were pharmaceutical grade.

2. Instrumentation

Experiments were conducted by Agilent Model 7890A gas chromatograph associated with GC sampler 80. Headspace sampling was carried out using a 2.5 ml headspace syringe with a 23 gauge pt no. 5 needle. Carryover in the headspace syringe was eliminated by an automatic syringe flush performed after each injection.A 30 m × 0.25 mm i.d. ZB-WAX (100% PEG) column with 0.25 µm film thickness (Phenomenex, USA) was utilized for gas chromatographic separation.

3. SHS-GC-FID instrumental conditions

The headspace sampling parameters were as follows: incubation temperature: 60°C; incubation time: 30 min; syringe temperature: 65°C; agitation speed: 500 rpm; syringe injection volume: 625 µl.

The gas chromatographic conditions were as follows: the carrier gas was helium (99.999%) at a constant flow rate of 0.9 mL/min; the injector was maintained at 170 °C with a split ratio of 1:25; the FID was set at 280 °C; the column oven temperature program involved an initial temperature of 35 °C for 5 min, increased at 40 °C/min to 220 °C and held for 1 min.

3. Formic acid derivatization

Formic acid was derivatized to ethyl formate using 1% (w/w) *p*-toluenesulfonic acidified ethanol as diluent and derivatization reagent. The derivatization reaction was carried out in the headspace vial which was heated at 60°C for 30 min in the headspace incubator.

4. Sample preparation

A 250 mg of the sample was directly weighted into a 20 ml headspace vial. After that, 5 mL of 1% (w/w) *p*-toluenesulfonic acidified ethanol solution was added to the vial which then was immediately sealed with a magnetic screw cap lined with a butyl/PTFE septum and sonicated till being completely dissolved.

5. Standards preparation

The concentration of formic acid, used in experiments,was determined by the acidimetric method described in British Pharmacopeia 2013 [15]. It was 99.54% (w/w).

Standard solutions of formic acid were prepared in acidified ethanol. A stock standard solution at 1264.16 µg/ml of formic acid was used to prepare a series of standard solutions. Then, five milliliters of each standard solution were transferred to each headspace vial.

Results and Discussion

1. Development of SHS-GC-FID method

1.1. Chromatographic conditions

1.1.1. Identification

To confirm the identity of the resultant derivative (formic acid derivatized with ethanol), the retention time of formic acid derivative was compared with that of the corresponding standard (ethyl formate). Results showed that there were complete matching between the retention times of both formic acid derivative and the corresponding standard. This confirms the identity of the resultant derivative as it is ethyl formate.

1.2. Determination of Incubation Time

To determine the proper incubation time for complete the derivatization reaction of formic acid, four incubation times (15, 30, 45 and 60 min) of the spiked samples were evaluated. Fig. 1 shows the diagram which correlates between the peak area of the derivative and the increased incubation time. Results demonstrated that there was no increasing in the peak area of formic acid derivative (ethyl formate) after incubation for 30 min in both PEG 400 and PVP K-30. So, the incubation at 60 °C for 30 min was sufficient to complete the derivatization reaction and to reach the equilibration of the derivative between the sample and gas phases.

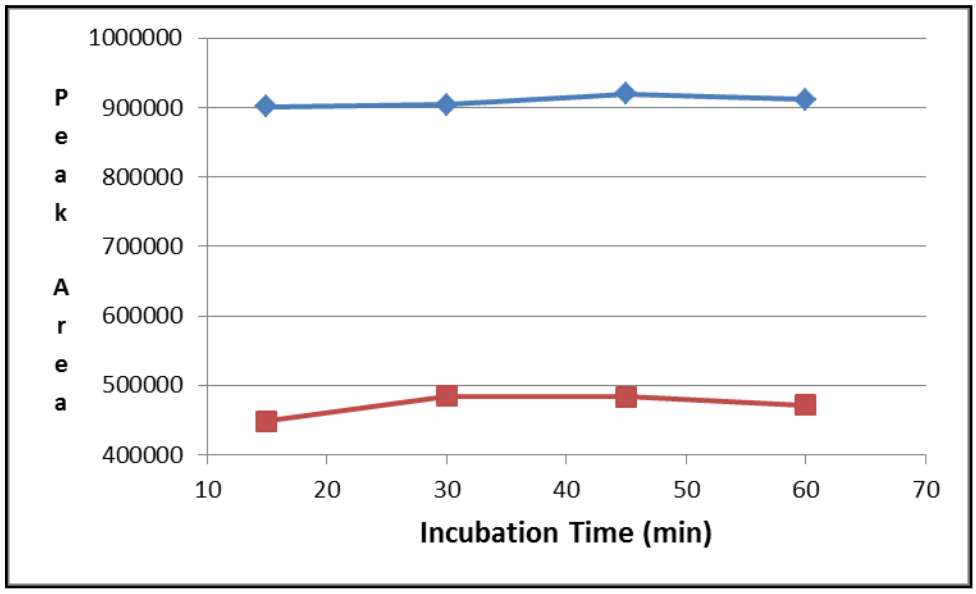

Fig. 1.Effect of incubation time at 60° C on formic acid derivative peak area. (■)in PEG 400 and (♦)in PVP K-30.

Table 1 Excipients sample analysis (n=2)

Source	Excipient	Formic acid Level (µg/g)
A	PVP K-30	2014.18
B	PVP K-30	59.55
C	PVP K-30	1188.30
D	PVP K-25	820.63
A	PEG 400	107.08
C	PEG 400	9.00
E	PEG 300	3265.10

2. Method Validation

2.1. Specificity

Specificity of the method for estimation of formic acid in excipient samples was proven that none of the peaks that belong to the samples coeluted with formic acid derivative. It was also found that there was no interference from the blank as well.

2.2. Linearity

The linearity of the method was evaluated from triplicate injections of six concentrations at the following range: 0.253-50.566 µg/mL. The analyte showed perfect linear behavior over the specified range with coefficient of correlation (r^2) value of 0.99995.

2.3. Accuracy and Precision

The accuracy (mean recovery) and repeatability(intra-day precision) of the method were evaluated at four concentration levels of formic acid (25.28-503.87-756.5-1011.33 µg/g excipient sample)in triplicate. Intermediate precision (inter-day precision) was estimated by analyzing the spiked excipient samples on two different days by two analysts. At all levels the mean recovery was within the acceptable range (80-120%) and RSD was less than 15%.

2.4. Limit of detection

The detection limit was determined based on the SD of the y-intercept and the slope (SL) of the linearity curve (LOD = 3.3*SD/SL). It was 127.15µg/L. The detection limit in excipient samples were 2.54 µg/g because the sample concentration is 50 mg/mL.

2.5. Limit of quantitation

The quantitation limit was determined based on (LOQ = 10*SD/SL). It was385.29 µg/l(7.71µg/g in excipient samples).

2. Pharmaceutical excipient samples analysis

Table 1 shows the results of analysis of formic acid in PEGs and PVP samples. The formic acid levels in the tested samples were extensively varied from 9.00to 3265.10 µg/g. These results depend on the nature, the storage conditions and the source of the excipients.

All PVP samples had lower levels of formic acid than the pharmacopeial limit (0.5%) [15].However, most of them contained high levels of formic acid which can lead to critical drug products instability issues. PVP K-30 from the source B had the lowest level of formic acid.

Although PEGs monographs do not require a limit test for formic acid, but the excipient samples contained considerable levels of it. PEG 300 had the highest level of formic acid(3265.10 µg/g)among all the tested samples. Such level could lead to both toxicity and drug products instability, especially if the formulation process results in formation of more formic acid or if other pharmaceutical ingredients have some levels of this impurity. This would be more critical if the dosage form is parenteral [3, 17].

Finally, trace levels of reactive impurities may cause important drug products degradation, especially when excipient/active pharmaceutical ingredient ratio is high [1, 4].

Conclusion

A SHS-GC-FID method was developed and validated for determination of formic acid in pharmaceutical excipients. Excipient samples were simply prepared in headspace vials using acidified ethanol as a diluent and derivatization reagent. The method was linear, specific, accurate and precise. The limits of detection and quantitation in excipient samples were2.54 and 7.71µg/g, respectively. The method was used to screen the low grades of PVP and PEGs samples for presence of formic acid. The tested samples contained

different levels of this impurity over a wide range. So, the developed method can play an important role in selecting appropriate vendors, excipients and/or excipient batches for pharmaceutical formulation. It can also be applied as a tool for testing the quality of pharmaceutical excipients routinely.

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