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Simultaneous determination of formic acid and formaldehyde in pharmaceutical excipients using headspace GC/MS

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Abstract

Formic acid and its esters, as well as formaldehyde, are trace impurities that are often present in pharmaceutical excipients. These trace impurities can potentially react with amino and/or hydroxyl groups in drugs to form significant levels of degradants. To select the appropriate excipients for a stable formulation, a gas chromatography/mass spectrometry (GC/MS) method was developed and validated for the rapid screening of trace amounts of residual formic acid, its esters and formaldehyde in pharmaceutical excipients. Samples were dissolved or dispersed in acidified ethanol to convert formic acid and formaldehyde to ethyl formate and diethoxymethane, respectively. Identification was conducted using a GC/MS system under scan mode and quantified using a selected ion monitoring (SIM) mode. Evaluation of the mass spectra of ethyl formate and diethoxymethane in the samples indicated that the method is specific. The limits of quantitation of the method were 0.5 ppm for formic acid and 0.2 ppm for formaldehyde. The precision of the method was demonstrated by the acceptable R.S.D. ($\leq$10%) over a linear range of 0.5–10,000 ppm. The accuracy of the method was within 80–120% over the linearity range. The amounts of formic acid and formaldehyde in commonly used pharmaceutical excipients is reported.

Keywords: Formic acid; Formaldehyde; Pharmaceutical excipients

1. Introduction

During early drug formulation development, excipients are generally screened under accelerated and stressed conditions to determine their chemical and physical compatibility in mixtures with drug substances. Excipients that react chemically with the drug and result in an increase in the levels of degradation products are typically not used for further formulation development [1–5]. This decrease in the stability of the drug substance during the compatibility studies is often attributed to trace levels of impurities present in the excipients studied. To date, little effort has been made to identify these impurities due to the complexity of these studies [6,7].

Formic acid is present at low levels in some excipients but is generally not tested or specified by the excipient manufacturers due to the low toxicity of this class III solvent [8]. Formic acid may be present as an ester in excipients that have hydroxyl groups or alcohol impurities. Both formic acid and its esters are chemically reactive compounds that may interact with amino and/or hydroxyl functional groups in many pharmaceutical compounds to form the corresponding amides and/or esters as shown in Fig. 1.

Formaldehyde is a common residual impurity in many excipients such as polysorbate, povidone and polyethylene glycol 300 [9]. The presence of this impurity can potentially decrease the stability of drug substances by reacting with the amino group to form the *N*-methyl derivative [10] as shown in Fig. 1. In addition, formaldehyde is known to crosslink gelatin which causes incomplete capsule shell dissolution in vitro and subsequent drug release problems [11].

Formaldehyde is susceptible to oxidation and is partially converted to formic acid on contact with air [12]. Therefore, excipients having residual formaldehyde are expected to contain some formic acid. Since both of these impurities can coexist

Abbreviations: FID, flame ionization detector; GC/MS, gas chromatography/mass spectrometry; HPMC, high performance liquid chromatography; HPMC, hydroxypropyl methylcellulose; LOQ, limit of quantitation; PEG 3500, polyethylene glycol 3500; PEG 4000, polyethylene glycol 4000; PEG 400, polyethylene glycol 400; SIM, selected ion monitoring; TIC, total ion count

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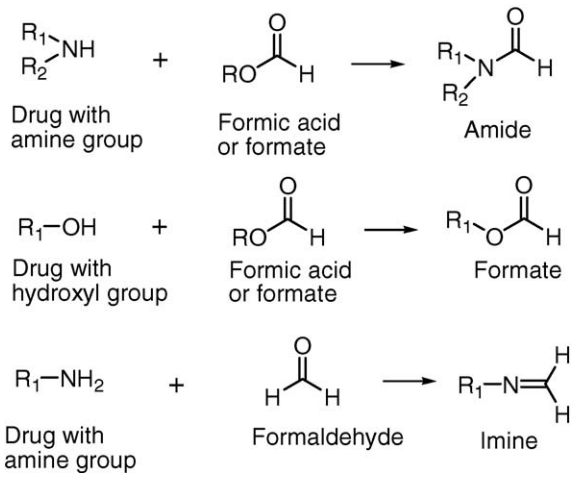

Fig. 1. Reaction scheme of formic acid, formate and formaldehyde with drugs having amino and/or hydroxyl groups.

in many excipients and react with the drug substances to affect stability of the drug products, it is necessary to develop a rapid, sensitive and reliable analytical method to simultaneously determine formaldehyde, formic acid and formic acid esters.

To our knowledge, there have been no methods reported in the literature for simultaneous determination of trace amounts of formaldehyde, formic acid and formic acid esters in pharmaceutical excipients. In this study we have developed and validated a rapid and sensitive gas chromatography method to simultaneously determine the formic acid and its esters as well as formaldehyde in pharmaceutical excipients. This method can be utilized for screening various excipients to be used in the development of stable formulations for drug substances that are susceptible to the reactions with formaldehyde, formic acid and formic acid esters.

2. Experimental

2.1. Reagents and chemicals

The solvents used were ACS grade. Ethyl alcohol (200 proof) and formic acid were purchased from EM Science and the 37% formaldehyde solution was from Sigma. *p*-Toluenesulfonic acid was purchased from EMD Chemicals and sulfuric acid from Alfa Aesar. Excipients used in this study were purchased from the following vendors: lactose (Quest International Inc.), powdered cellulose (JRS), mannitol (SPI Pharma Inc.), microcrystalline cellulose of various particle size (FMC), Starch 1500 (Colorcon), food starch (Cerestar), dibasic calcium phosphate (Penwest Pharmaceuticals Co.), Povidone K-25 and 90-F (BASF), Plasdone K-25 (ISP Technologies), hydroxypropyl methylcellulose (Dow), Polyplasdone XL (ISP Technologies), croscarmellose sodium (FMC), sodium starch glycolate (Penwest Pharmaceuticals Co.), magnesium stearate (Mallinckrodt, Inc.), stearic acid (WITCO Corp.), glycerol dibehenate (Gattefosse), polyethylene glycol 3500 and 4000 (Union Carbide Corp.), polyethylene glycol 400 (Union Carbide Corp.) and sodium dodecyl sulfate (J.T. Baker).

2.2. Gas chromatography/mass spectrometry (GC/MS) conditions

Experiments were performed on an Agilent Model 6890N gas chromatograph equipped with a Model 5973N quadrupole mass selective detector (MSD) and Model 7694 headspace autosampling unit. Separation was achieved using a Phenomenex ZB-WAX column (100% polyethylene glycol), 30 m long with a 0.32 mm i.d. and 0.5 μm film thickness. The carrier gas was helium and was set at a constant flow rate of 2.5 mL/min (57 cm/s) with a head pressure of 6.35 psi. The injector was maintained at 170 °C with a split ratio of 10:1 and split flow of 25 mL/min. The headspace sample and standard solutions were equilibrated at 60 °C for 15 min. The vials were pressurized for 6 s, sample loop filled with the headspace gas for 30 s and equilibrated for 3 s before a 1 mL injection was taken. The loop and transfer line was set at 120 °C. The column oven temperature program was set at 40 °C initially for 4.2 min, increased at 40 °C/min to 200 °C and held at 200 °C for 2 min. Mass selective detector detection was performed at 280 °C with either full scan (25–150 amu) for identification or with selected ion monitoring (SIM) mode for quantitative analysis. The qualifying ions for SIM mode were *m/z* 27 and 31 for ethyl formate and *m/z* 31, 59 and 103 for diethoxymethane. Chromatographic data were collected and evaluated using MSD Productivity Chemstation Software.

2.3. Sample preparation

Sample solutions were prepared by accurately weighing approximately 500 mg of the excipient into a 20 mL headspace vial and dissolving or dispersing it in 5 mL of the acidified ethanol solvent (1% *p*-toluenesulfonic acid). Samples were immediately sealed with an aluminum crimp cap lined with a teflon/butyl septum and sonicated for 1 min for adequate dissolving or dispersing of the mixture. Prior to the injections, the completion of the derivatization of formic acid and formaldehyde in the sample solutions was achieved by heating the sample mixtures at 60 °C for 15 min under continuous agitation in the headspace autosampler.

2.4. Standard preparation

Standard solutions of formic acid and formaldehyde were prepared in acidified ethanol (1% *p*-toluenesulfonic acid). A stock standard solution at 1000 μg/mL (10,000 ppm relative to sample) was prepared and used to prepare standard solutions at lower concentration by serial dilutions. The concentration range used for linearity experiments was 0.02–1000 μg/mL (0.2–10,000 ppm). A total of 11 different concentration levels ($n = 3$) were used in the linearity experiment. Five milliliters of each standard solution were transferred to headspace vials and immediately sealed with an aluminum crimp cap lined with a teflon/butyl septum. Prior to the injections, the derivatization of formic acid and formaldehyde in the standard solutions were completed by heating the solutions at 60 °C for 15 min under continuous agitation in the headspace autosampler.

3. Results and discussion

3.1. Method development

In early developmental work, the challenges faced in direct determination of formic acid and formaldehyde using gas chromatography techniques were the poor recovery, peak shape and separation. This was mainly due to their high reactivity as well as the low response using flame ionization detection (FID) to the already oxidized carbon in their molecules [13]. These challenges can generally be overcome by the derivatization and subsequent determination of their derivatives using GC or high performance liquid chromatography (HPLC) techniques. Various derivatization methods followed by GC or HPLC determination of formic acid or formaldehyde have been reported [14–22] but the sample preparation generally involves complex chemical reactions and the methods are not suitable for the simultaneous determination of formic acid and formaldehyde in excipient matrices. A critical step in the method development was to find a suitable derivatization reagent. We selected an alcohol as the reagent since both formic acid and formaldehyde can readily react with the alcohol in the presence of an acidic catalyst to give the corresponding ester and acetal, respectively. The ester and acetal are volatile and suitable for GC determination. In addition, the alcohol can act as a solvent to dissolve or disperse the excipients. The large excess amount of alcohol can also drive the completion of the derivatization reactions. This dual function of the alcohol acting as the reagent and solvent gives a very simple one-step procedure for the sample preparation.

To select a suitable alcohol as the reagent and solvent several different alcohols (methanol, ethanol, propanol and butanol) were evaluated. Ethanol was selected based on visual inspection of the chromatogram showing it was the cleanest and had the least interference during GC separations of the derivatized analytes. A study of two acidic catalysts (sulfuric acid and *p*-toluenesulfonic acid) showed that sulfuric acid interfered with the quantitation of formaldehyde (Fig. 2). In contrast to sulfuric acid, *p*-toluenesulfonic acid did not give any interference. Based on these results, ethanol was selected as the derivatization

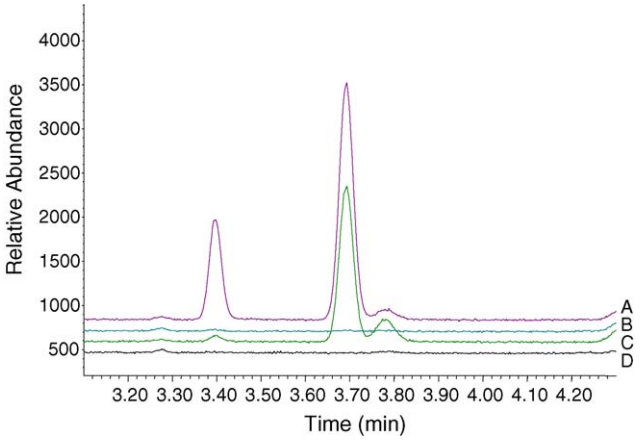

Fig. 2. Comparison of acid catalysts. (A) Formic acid and formaldehyde standard solution, (B) 1% *p*-toluenesulfonic acid in ethanol, (C) 1% sulfuric acid in ethanol, (D) ethanol only.

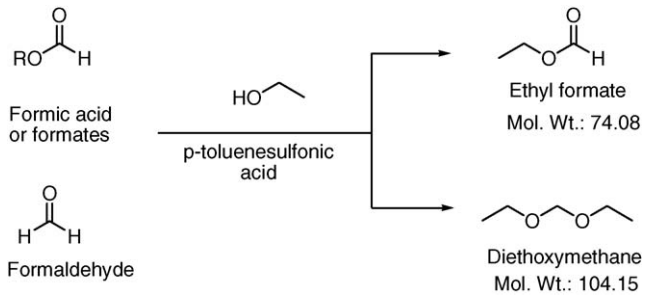

Fig. 3. Derivatization of formic acid to ethyl formate and formaldehyde to diethoxymethane in excipient samples and standards.

reagent and solvent and *p*-toluenesulfonic acid as the catalyst. The derivatization reactions using ethanol are shown in Fig. 3. In the presence of *p*-toluenesulfonic acid as an acid catalyst and large excess amount of ethanol, formic acid or formates readily undergo esterification or transesterification to form ethyl formate. In the meantime, formaldehyde reacts with two equivalents of ethanol undergoes acid-catalyzed acetal formation to give an acetal, namely diethoxymethane. A solution of 1% *p*-toluenesulfonic acid in ethanol was used in the method since it was found to be sufficient to provide rapid derivatization of the two analytes.

To optimize the derivatization reaction prior to GC injection, different heating temperatures (40, 50 and 60 °C) and lengths of heating times (5, 10, 15, 20 and 30 min) of the samples were evaluated. A heating temperature of 60 °C was selected because of the enhanced rate of the derivatization reaction compared to 40 and 50 °C. Heating temperatures higher than 60 °C were not tested to avoid potential degradation of excipients during the sample preparation. The length of heating time at 60 °C was determined based on the amount of ethyl formate and diethoxymethane formed during increased heating time of excipient samples. Complete derivatization of both analytes was indicated by the plateau of the peak response of ethyl formate and diethoxymethane with increase in heating time. Fig. 4 shows an example of this optimization based on PEG 400 sample analysis. The data show no significant increase in the response of the ethyl formate and diethoxymethane peaks after 15 min heating at 60 °C. This indicates that 15 min was sufficient time to

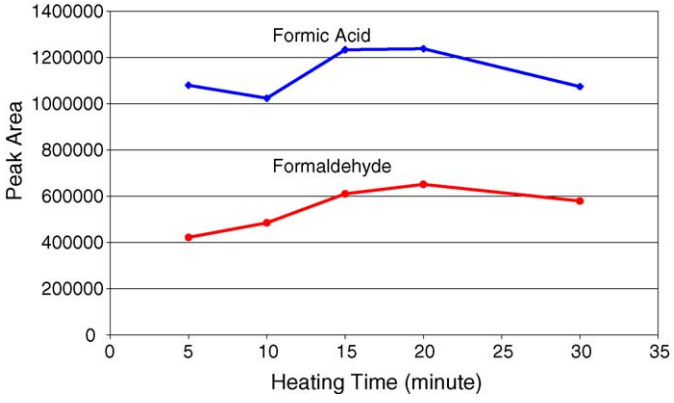

Fig. 4. Effect of heating time at 60 °C on peak area response of formic acid and formaldehyde in PEG 400, $n = 2$.

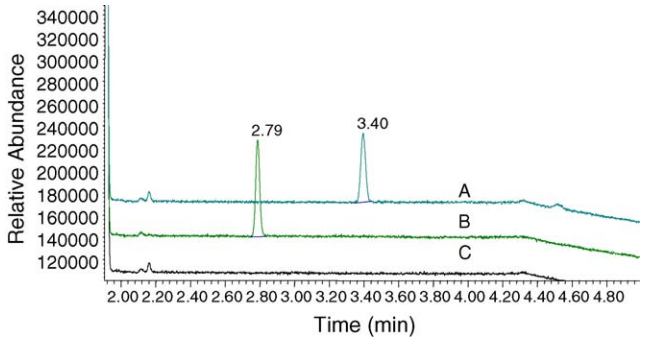

Fig. 5. The Total ion count (TIC) of the GC/MS analysis of methyl formate in acidified ethanol (A), methyl formate in ethanol (B) and the acidified ethanol blank (C). *Note*: The peak eluting at 2.79 min is methyl formate and the peak eluting at 3.40 min is ethyl formate.

completely carry out the derivatization reactions. Analysis of several other excipients showed the same response profile with the heating time. In a few instances, such as the analysis of hydroxypropyl methyl cellulose, the excipient did not evenly disperse during sample preparation and a longer heating time of 30 min was required to get complete derivatization.

The derivatization reactions were found to be reversible in the presence of water. To determine the tolerance of the derivatization of formic acid and formaldehyde to the presence of water, water was added at the level of 10, 50 and 100 mg/mL (10, 50 and 100% relative to sample concentration), in the derivatization reaction of excipient samples. The results showed that the presence of water at less than 10 mg/mL had little or no adverse effect on the derivatization reaction of both analytes. Excipient samples containing water content higher than 10 mg/mL (>10% relative to sample concentration) had slightly lower formic acid and formaldehyde conversion, but this problem was easily prevented by decreasing the sample concentration of the excipient to reduce the water concentration below 10 mg/mL in the derivatized sample.

Formic acid may occur as its ester forms in the excipients containing hydroxyl groups, such as lactose, starch, PEG and cellulose, while it occurs as the free formic acid form in non-hydroxyl excipients. In addition to the determination of free formic acid and formaldehyde, we extended the GC/MS analysis to include other formic acid esters or formates in this method since these esters in excipients can also potentially react with drugs that have hydroxyl or amino groups. It is expected that these esters will be readily converted to ethyl formate through transesterification with ethanol under the derivatization conditions (Fig. 3). Since these esters are complex and not available to us, we used methyl formate as a model ester to confirm this expected conversion. Fig. 5 shows the comparison of chromatograms between methyl formate added in ethanol and methyl formate added in the sample solvent (1% *p*-toluenesulfonic acid in ethanol). The results show the full conversion of methyl formate to ethyl formate and suggest that formic acid esters in the excipients can be readily converted to ethyl formate. Thus, formate esters are included as part of the formic acid amount determined and reported in this method.

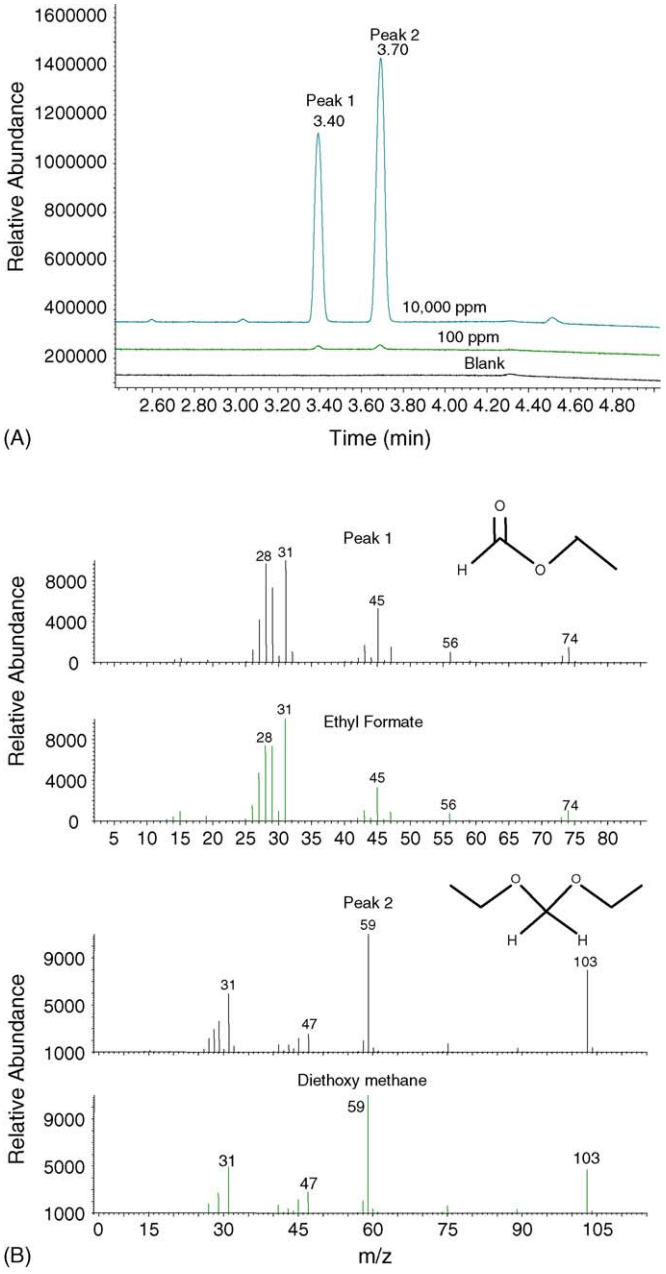

Fig. 6. (A) TIC of the GC/MS analysis of the standard of formic acid and formaldehyde in scan mode. (B) Electron impact mass spectra of ethyl formate and diethoxymethane in the standard solution. Peak 1: ethyl formate; Peak 2: diethoxymethane.

Fig. 6A illustrates typical GC/MS chromatograms of blank (1% *p*-toluensulfonic acid in ethanol) and formic acid and formaldehyde standards at 100 and 10,000 ppm under scan mode using the optimized sample preparation procedure. Comparison of the blank and standard solutions show two new peaks in the standard solutions eluted as sharp, well defined peaks at 3.4 min (Peak 1) and 3.7 min (Peak 2). The mass spectra of Peak 1 and Peak 2 as shown in Fig. 6B were identified using the NIST MS library [23] as ethyl formate and diethoxymethane, respectively, the derivatized reaction products. The blank solvent was clean with no significant interfering peaks at the retention times of ethyl formate and diethoxymethane.

Table 1
Recoveries after spiking at 50 ppm level of formic acid and formaldehyde ($n = 3$)

Major use	Excipient	Recovery (%)		Intra-day precision (%)		Inter-day precision (%)	
		Formic acid	Formaldehyde	Formic acid	Formaldehyde	Formic acid	Formaldehyde
Filler	Lactose	96.4	99.4	2.2	0.8	2.7	3.3
Filler	Microcrystalline cellulose, 50 μm	90.2	95.8	2.2	1.9	1.9	2.1
Filler	Starch 1500	93.5	94.4	1.3	2.1	1.6	1.3
Binder	Hydroxypropyl methylcellulose	87.4	105.5	3.7	11.1	3.6	1.8
Disintegrant	Polyplasdone XL	97.4	101.5	2.0	1.1	3.7	3.6
Disintegrant	Sodium starch glycolate	95.5	99.1	6.2	2.8	3.0	2.4
Lubricant	PEG 4000	98.1	100.9	1.6	0.8	1.1	0.9
Lubricant	Stearic acid	91.8	87.5	1.2	1.5	3.4	3.0

In order to improve the sensitivity of the MS detection, the selected ion monitoring mode was used. At least two prominent fragment ion peaks, not present in the background, were selected for each derivatized product. Using the SIM mode, the performance characteristics of the GC/MS method were evaluated with respect to linearity, range, detection limit, precision and accuracy, and this mode was subsequently used in the screening of pharmaceutical excipients.

3.2. Method validation

The specificity of the method in determining ethyl formate and diethoxymethane was demonstrated by the well-defined GC separation of the two analytes as shown in Fig. 6. The specificity of the method was confirmed by both the positive identification of the derivitization products by their mass spectra and obtaining a solvent blank chromatogram free of co-eluting peaks at the retention times of ethyl formate and diethoxymethane.

The linearity of the method was evaluated from triplicate injections of a series of standard solutions over the concentration range of 0.2–10,000 ppm for each analyte. The quantitative range of the method was 0.5–10,000 ppm for formic acid and 0.2–10,000 ppm for formaldehyde. The coefficients of correlation (r^2) of the standard curves were 0.9937 and 0.9960 for formic acid and formaldehyde, respectively. The average relative standard deviation for specific concentrations on the standard curves of formic acid and formaldehyde were 3.1 and 4.8%,

Table 2
Formic acid and formaldehyde levels in pharmaceutical excipients ($n = 3$)

Major use	Excipient	Manufacturer	Lot	Level (ppm)	
				Formic acid	Formaldehyde
Filler	Lactose	A	1	1.0	<0.2
Filler	Powdered cellulose	B	1	2.9	0.4
Filler	Mannitol	C	1	0.0	0
Filler	Microcrystalline cellulose, 50 μm	D	1	9.3	<0.2
Filler	Microcrystalline cellulose, 100 μm	D	2	23.9	0.9
Filler	Microcrystalline cellulose, 100 μm	D	3	11.8	1
Filler	Microcrystalline cellulose, 180 μm	D	4	4.0	0.3
Filler	Starch 1500	E	1	3.0	<0.2
Filler	Starch	F	1	2.4	0
Filler	Starch	F	2	5.1	0
Filler	Dibasic calcium phosphate	G	1	1.5	0
Binder	Povidone K-25	H	1	3080.3	<0.2
Binder	Povidone 90	H	2	630.7	0.4
Binder	Povidone K-25	I	1	1990.5	0.4
Binder	Hydroxypropyl methylcellulose	J	1	58.3	11.1
Binder	Hydroxypropyl methylcellulose	J	2	86.4	15.7
Disintegrant	Polyplasdone XL	I	1	10.8	0
Disintegrant	Croscarmellose sodium	D	5	26.3	0
Disintegrant	Sodium starch glycolate	G	2	1.9	0.9
Lubricant	Magnesium stearate	K	1	2.1	1.1
Lubricant	Stearic acid	L	1	0.0	0
Lubricant	Glycerol dibehenate	M	1	4.3	2.1
Lubricant	Polyethylene glycol 3500	N	1	2.3	0.3
Lubricant	Polyethylene glycol 3500	N	2	1.7	1.2
Lubricant	Polyethylene glycol 4000	O	1	1.7	0.9
Lubricant	Polyethylene glycol 4000	N	3	14.0	3.6
Solvent	Polyethylene glycol 400	O	2	469.0	85.8
Surfactant	Sodium dodecyl sulfate	P	1	0.0	0

respectively, with values ranging from 1.3 to 6.2% and from 1.3 to 10.1%.

The limit of quantification (LOQ) of the method was defined as the lowest concentration of the analyte in the sample that can be determined with acceptable precision (≤10% R.S.D.). The LOQ of formic acid was 0.5 ppm and that for formaldehyde was 0.2 ppm. The relative standard deviations obtained from the results of six consecutive injections of the standard solution at the LOQ levels was 8.9% (formic acid) and 7.5% (formaldehyde).

The accuracy and inter- and intra-day precision of the method are shown in Table 1. The data were generated based on a single level recovery study of both analytes in eight selected excipients ($n = 3$) analyzed on two different days by two different investigators. The accuracy values varied from 87.4% (HPMC) to 98.1% (PEG 4000) for formic acid and from 87.5% (stearic acid) to 105.5% (HPMC) for formaldehyde. The corresponding intra-day precisions were from 1.2% (stearic acid) to 2.6% (Explotab) for formic acid and from 0.8% (PEG 400) to 11.1% (HPMC) for formaldehyde. The inter-day precision for each analyte was below 3.7% in each case. The good recoveries of the formic acid and formaldehyde (within 80–120%) indicate that the matrix effect was insignificant for the excipients tested.

The formic acid and formaldehyde contents in commonly used pharmaceutical excipients determined by this method are shown in Table 2. A total of 28 excipients covering a range of formulation functionality varying in grade, batch and/or vendor were screened. The data show that almost all the excipients contained some level of formic acid. Very few contain formaldehyde above 1 ppm level. The formic acid and formaldehyde levels in the excipients tested varied from 0 to 3080 ppm and 0 to 86 ppm, respectively. Povidone contained significantly higher levels of formic acid (3080.3 ppm) compared to other excipients analyzed. This amount varied depending on the grade and supplier. As expected, PEG 400 contained significant levels of both formic acid and formaldehyde (469 ppm of formic acid and 86 ppm of formaldehyde). It is likely that the formaldehyde content in freshly opened PEG 400 may be greater than 86 ppm and that the formaldehyde is oxidized over time to formic acid. Microcrystalline cellulose contained significantly higher levels of formic acid compared to other filler excipients analyzed. The level varied depending on the grade and the lot.

4. Conclusions

A new and sensitive GC/MS headspace method has been developed and validated for the rapid and simultaneous determination of trace levels of formic acid and its esters, as well as formaldehyde in pharmaceutical excipients. Sample preparation was optimized to provide a simple one-step procedure requiring dissolving or dispersion of samples in acidified ethanol reagent to convert formic acid and its esters to ethyl formate and formaldehyde to diethoxymethane. The GC/MS method has been demonstrated to be specific for both derivatized analytes and has been validated over the range of 0.5–10,000 ppm for formic acid and 0.2–10,000 ppm for formaldehyde. The limits of quantification were determined to be 0.5 and 0.2 ppm for formic acid and formaldehyde, respectively. The method showed good accuracy (80–120%) and intra- and inter-day precision (≤10%). Using this method, it was found that almost all excipients contained varying levels of formic acid and formaldehyde. Based on these findings, screening of excipients for formic acid and formaldehyde could be useful in selecting appropriate excipients and in selecting vendors or batches of excipients that contain low levels of the two impurities. This screening method would be important for formulation development and product quality control for drugs that are susceptible to the formation of amides, esters or imines.

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