

Gas Chromatography/  
Mass Spectrometry

## Author

Padmaja Prabhu

PerkinElmer, Inc.  
Shelton, CT USA

## Detection and Quantification of Formaldehyde by Derivatization with Pentafluorobenzylhydroxyl Amine in Pharmaceutical Excipients by Static Headspace GC/MS

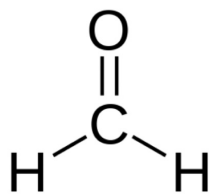


Figure 1. Structure and properties of formaldehyde.

### Introduction

Although considered pharmacologically inert, pharmaceutical excipients have been shown to interact with active drug substances to affect the safety and efficacy of drug products.<sup>1</sup> Therefore, there is an increasing awareness of the necessity to understanding interactions between excipients and the active pharmaceutical ingredient (API) in finished dosage forms.

One of the areas of major concern is the potential chemical interaction between impurities in the excipient with the drug molecules, leading to formation of reaction products.<sup>2</sup> Even trace amounts of reactive impurities can cause significant drug stability problems as the quantity of excipients in a formulation often far exceeds that of an API on a weight and molar basis. Trace amounts of reaction products can then easily exceed 0.2% qualification thresholds for a degradation in many drug products.<sup>1</sup> Formaldehyde present in excipients has been implicated in the degradation of several drug products where it can form adducts with primary and/or secondary amine groups.<sup>2</sup> It has also been reported that formaldehyde can induce cross-linking in gelatin capsules causing an adverse effect on in-vitro dissolution rates of drugs. Because of the extremely high reactivity of aldehydes, a timely evaluation of their presence in excipients during formulation design is essential to avoid unexpected drug stability problems in later stages of product development.

The work presented here describes the development of a systematic approach for the detection and quantification of formaldehyde in excipients to provide additional insight to formulation development. Specifically, a static headspace GC (SHS-GC) method based on PFBHA derivatization and Electron Impact ionization (EI) Mass Spectrometry (MS) employing both scan and selected-ion monitoring (SIM) simultaneously was developed and employed to screen excipients for the presence of formaldehyde.

Formaldehyde (Figure 1) is a colorless gas with a characteristic pungent odor. It is a volatile organic compound having molecular weight of 30 amu and low boiling point (-21 °C). Because of its high volatility, formaldehyde levels in various matrices can be easily determined by headspace methods with a fair degree of accuracy.

## Experimental

The PerkinElmer® Clarus® 680 Gas Chromatograph, Clarus Mass Spectrometer and a TurboMatrix™ Headspace 40 system were used for this application. Tables 1, 2 and 3 present the detailed operating parameters of the GC/MS and the headspace system. The instrument interaction, data analysis and reporting was completed with the PerkinElmer TurboMass™ data system.

**Stock solution:** 50 mg of formaldehyde was weighed into a 100 mL flask and diluted to volume with bottled water to make 200 µg/mL mixture of formaldehyde.

**Solution A:** 5 mL of the stock solution was diluted to 100 mL with bottled water to give a concentration of 10 µg/mL.

**Solution B:** 5 mL of the mixture A was diluted to 100 mL with bottled water to give a concentration of 0.5 µg/mL.

**Internal standard solution (I.S.):** 50 mg of cyclohexanone was weighed into a 50 mL flask and diluted to volume with bottled water to make 1000 µg/mL. 10 mL of this solution was diluted to 250 mL with water and finally 20 mL of this was diluted to 1000 mL with water. This I.S. solution was used for dilution of calibration curve standards and preparation of solution.

**PFBHA solution:** 100 mg of PFBHA was dissolved in bottled water and 1 mL of this was added to each of the HS vials for derivatizing formaldehyde in samples and standard.

**Calibration curve:** Varying volumes of 0.5 µg/mL formaldehyde (solution B) was diluted in water to achieve the final standard concentration presented in Table 4. The solutions were made up to the final volume with I.S. solution. 1 mL of PFBHA solution was added to each of the vials for derivatization. 1 g of NaCl was added to each of the vials to decrease the miscibility of formaldehyde in water and enhance equilibration in the headspace.

**Table 1. Details of GC method.**

Instrument Details	Clarus 680 Gas Chromatograph
Analytical column	PerkinElmer Elite -5 MS (30 meter, 0.25 mm i.d., 0.25 µm df)
GC column flow	1.2 mL/min helium at constant flow mode
GC inlet temperature	190 °C
Split ratio	5:1
Oven temperature	50 °C hold for 3.0 min, 7 °C/min to 150 °C and hold for 5.0 min, 40 °C/min to 280 °C and hold for 5.0 min runtime is 20 min.

**Table 2. Details of HS method.**

Instrument Details	TurboMatrix HS-40	
Temperatures	Thermostatting	60 °C
	Needle	100 °C
	Transfer line	130 °C
Time	Injection	0.2 min
	Pressurization	0.5 min
	Withdrawal	0.2 min
	Equilibration	20 min
	Cycle	20 min
Options	Vial vent	ON
	Shaker	ON
	Operation mode	Constant
	Injection mode	Time
PPC	Hi psi injection	ON
	Inject	25 psi
	Column/headspace pressure	25 psi

**Table 3. Details of MS conditions.**

Instrument Details	Clarus Mass Spectrometer
Source temperature	200 °C
Interface temperature	200 °C
Scan range	m/z 40-380
SIM mode: ions monitored	178, 181, 197
Scan time	7.5-30 min
Multiplier	550

**Table 4. Scheme used for the creation of a five level calibration.**

Calibration level #	Concentration of formaldehyde in ppb	Standard solution added in mL	Final vol. (mL)
1	10	2 (from solution B)	100
2	20	4 (from solution B)	100
3	30	0.6 (from solution A)	100
4	40	0.8 (from solution A)	100
5	50	1.0 (from solution A)	100

\*1 g of NaCl was added to each of the headspace vials.

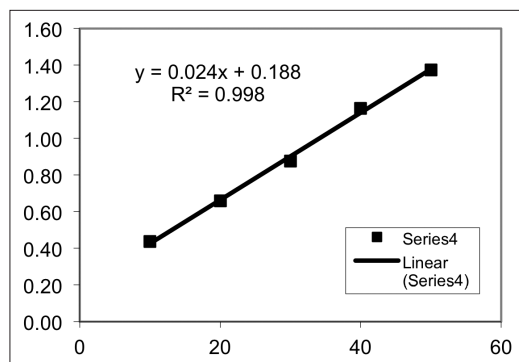


Figure 2. Calibration curve for formaldehyde.

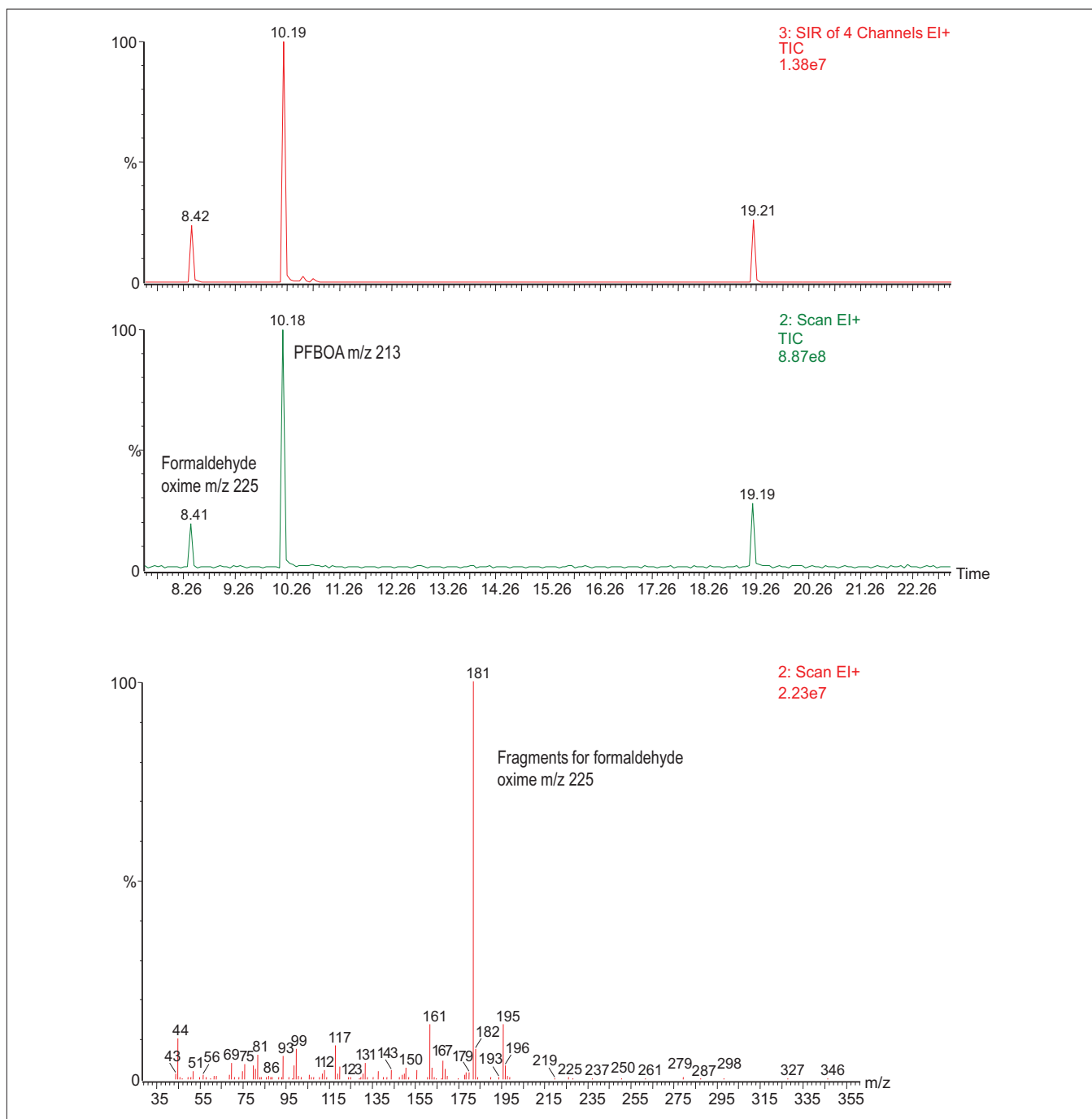


Figure 3. Example chromatogram and MS spectrum for formaldehyde in standard.

**Calibration:** The MS was calibrated across the range of 10.0 to 50 ppb of formaldehyde, each calibration point was run in triplicate to demonstrate the precision of the system. The calibration curve for formaldehyde is depicted in Figure 2. The average coefficient of determination for a line of linear regression was 0.998 for formaldehyde. Precision of the system across the calibration range is excellent. The percent relative standard deviation is shown in Table 5. Chromatograms and the spectrum from the analysis of standard material are shown in Figure 3. The peak at retention time 8.41 is that of formaldehyde oxime and the unreacted PFBOA elutes at 10.82 min.

**Table 5. % RSDs for three sets of linearity experiment.**

Concentration of formaldehyde in ppb	Mean peak area ratio average relative response (n=3)	%RSD
10	0.44	9.23
20	0.66	4.42
30	0.88	6.29
40	1.17	10.76
50	1.37	9.77

The precision of the method was measured at 5 ppb. The loss of precision at 5 ppb indicates the detection limit of this method to be approximately 5 ppb RSD 19.30%.

### Sample preparation

Plasdone, pregelatinised starch, povidone, polyethylene glycol (PEG-400), microcrystalline cellulose and lactose samples were obtained from one of the local pharmaceutical firms (Getz Pharma®, India). The headspace sample preparation is relatively very easy. A weighed sample (0.05 g to 0.2 g) was placed in a headspace vial and 10 mL of I.S. solution was added to each of the vials; 1 g of NaCl and 1 mL of PFBOA solution was added to each of the vials. The vials were incubated at the headspace conditions and analyzed. All the samples were freshly prepared and analyzed immediately.

### Method validation

The recovery of the method was tested with the analysis of the excipient sample spiked with formaldehyde at three different levels: 10, 20, 30 ppb (See Table 6). The recovery values are as shown in the results table and indicate the headspace technique is quantitative in its extraction of formaldehyde from an aqueous matrix.

**Table 6. Summary of method validation experiment**

Linearity:	10.0 ppb to 50 ppb of formaldehyde
RSD for replicate analysis:	for 10.0 ppb is 9.23%
Detection level:	5.0 ppb
Quantification level:	10.0 ppb
Recovery study:	at three levels for all the samples 80-120%

## Results and Discussion

Six samples of common excipients were analyzed using the SHS-GC/MS method developed here. These samples were chosen because they had been shown to have detectable levels of aldehydes in the literature. Of the samples analyzed, PEG-400 demonstrated to have the highest levels of formaldehyde at 3.5 ppm. Table 7 presents the results of the pharmaceutical sample study. The typical chromatogram and the spectrum from the analysis of samples material is shown in Figure 4.

**Table 7. Results of pharmaceutical study.**

Sample number	Sample details	Amt. of formaldehyde found in ppm	Recovery
1	Plasdone	2.2	81.37-108.66
2	Pregelatinised starch	1.2	91.33-112.86
3	Povidone	0.5	92.43-95.34
4	PEG-400	3.5	76.63-111.15
5	Microcrystalline cellulose (MCC)	0.3	93.28-104.46
6	Lactose monohydrate	0.5	99.16-104.46

Prior to the selection of an analytical technique for the determination of low-molecular-weight formaldehyde in excipients, a target level of method sensitivity (detection limit) with regard to excipient reactivity was considered. As the exact correlation between the aldehyde content and their reactivity with the pharmaceutical product is not known, and is a case-by-case relationship in pharmaceutical formulations, a worst case scenario was assumed to determine the desired limit of detection. It is not uncommon that the weight ratio of excipients to API in formulations exceeds 100:1.<sup>1</sup> For a small molecule API with a molecular weight of 500, the presence of aldehydes at 1 µg/g in excipients may result in a level of formaldehyde adduct as high as 0.2%, the lowest qualification threshold specified in the ICH guideline.<sup>3</sup> Therefore, 1 µg/g was thought to be reasonable as a threshold for the total content of low-molecular-weight aldehydes in excipients.<sup>1</sup>

One of the primary advantages of PFBHA derivatization is that it can be done in aqueous solution, desirable for headspace analysis as water generally provides very clean backgrounds.

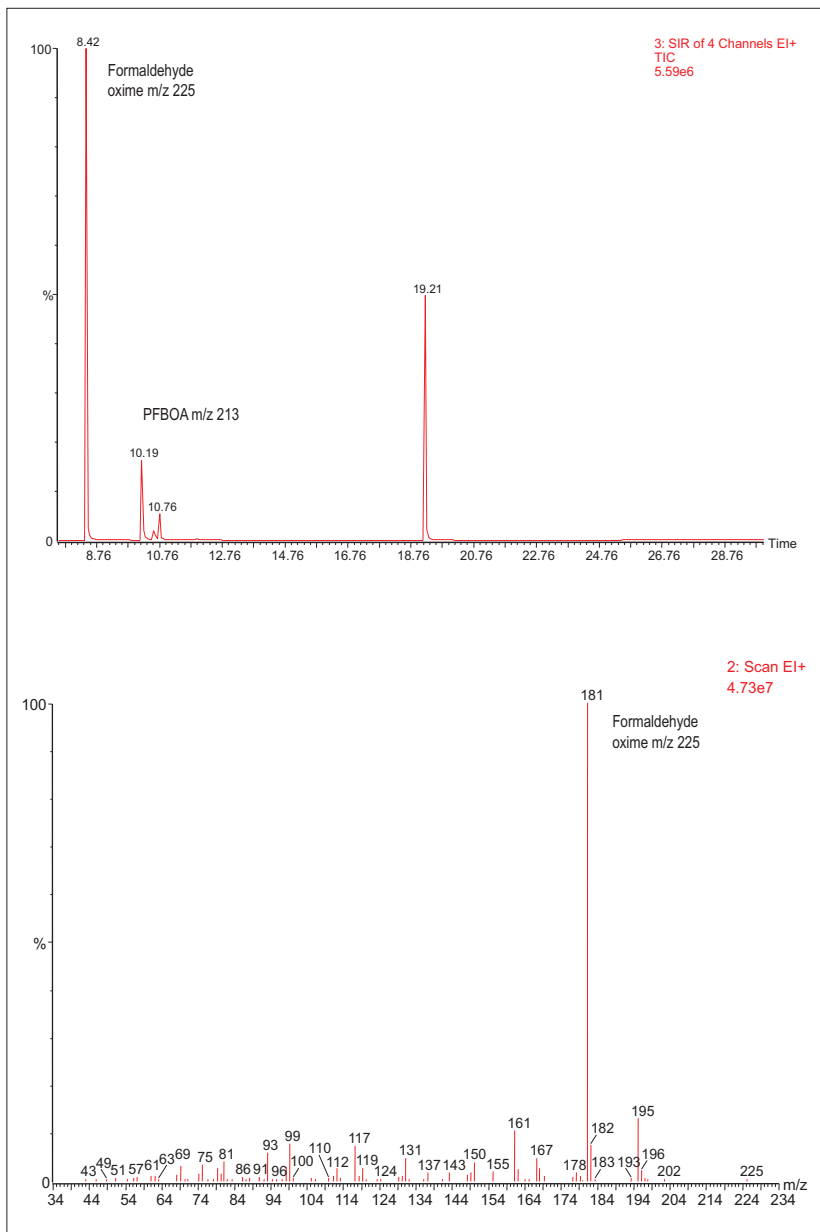


Figure 4. Chromatogram and spectrum of formaldehyde peak in sample.

## Conclusion

This application note presents a simple and effective method for the determination of formaldehyde in pharmaceutical excipients using SHS-GC/MS. The method is fast, reliable and can be used for the quantification of low-molecular-weight aldehydes in most excipients commonly used in pharmaceutical products. Excellent quantification and linear instrument response was reported across a 1 to 50 ppb concentration range of formaldehyde. The method was validated using several samples obtained from a local pharmaceutical company and observed recovery values were all between 80-120%. By combining GC with MS, formaldehyde oxime was identifiable not only through retention time matching but by the resulting mass spectrum, which was confirmed by library search.

## References

1. Excipient Toxicity and Safety, M.L. Weiner, L.A. Kotkoskie, Marcel Dekker, New York, 1999.
2. Journal of Chromatography, Volume A 1104, Issues 1-2, Pages 1-384 (February 2006), Detection and Quantification of Low-molecular Weight Aldehydes in Pharmaceutical Excipients by Headspace Gas Chromatography, Zhong Li, Laura K. Jacobus, W. Peter Wuelfing, Mary Golden, Gregory P. Martin and Robert A. Reed.
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