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ASUNTO                      Radicación                      09-002495  
                                         Trámite                              11  
                                         Evento                             378  
                                         Actuación                        519  
                                         Oficio  
                                         Folios                              7                      **E 1 4 5 9 3**

Patente de Invención                      ☒                      Patente de Modelo de Utilidad

Vía Nacional

Vía PCT (Fase Nacional)                      ☒ Capítulo I                      ☒ Capítulo II

No. Solicitud Internacional                      PCT/US2007/015771

Fecha de Presentación Internacional                      11/Julio/2007

Como resultado del Examen de Fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

|                            |                  |                         |
|----------------------------|------------------|-------------------------|
| Descripción:               | Folios 33 a 56   | 14/Enero/2009           |
| Reivindicaciones:          | Folios 57 a 66   | 14/Enero/2009           |
|                            | Folios 177 a 181 | 01/Abril/2001           |
| Oposiciones:               | Folios 107 a 161 | Procaps 13/Octubre/2010 |
| Respuesta del solicitante: | Folios 172 a 181 | 01/Abril/2001           |
| Prioridad:                 | US 11 487 120    | 14/Julio/2006           |

2. Análisis de la Prioridad

Se acepta la reivindicación de Prioridad porque esta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

Título de la solicitud: "COMPOSICIONES LIQUIDAS DE FENILEFRINA DE  
Expediente 09-2495                      1er Art 45

## ESTABILIDAD MEJORADA"

El problema planteado en esta solicitud consiste en la necesidad de proporcionar una composición líquida agradable que comprende fenilefrina con propensión reducida a la degradación de fenilefrina.

Para resolver este problema técnico la solicitud en estudio proporciona una composición oral líquida que comprende fenilefrina y sustancialmente polietilenglicol libre de aldehído.

El objeto de la presente Solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): A 61 K 9/00 // 31/137

### 4. Excepción a la Patentabilidad (Art 20 D 486 literal (d))

El objeto de las reivindicaciones 11, 37 -39 no es patentable de acuerdo con el Art citado.

### 5. De la Solicitud de Patente

#### Reivindicaciones (Art 30 D 486, Art 22 PCT)

La reivindicación 1 no es clara cuando se utilizan los términos "sustancialmente" y "al menos" los cuales no son limitativos ni precisos y no permiten distinguir la invención del estado de la técnica. Se sugiere sustituirlos por términos precisos o rangos concretos. Igual observación aplica para las reivindicaciones 2, 12 y 30

La reivindicación 3 no es clara cuando reclama una composición de la reivindicación 1 o de la reivindicación 2 que comprende adicionalmente al menos un segundo agente activo seleccionado del grupo que consiste de agentes analgésicos, descongestionantes, expectorantes, antitusivos, antipiréticos, antiinflamatorios, supresores de tos y adhistaminas. Esta manera de reclamar dicho componente no es válida puesto que no define un componente como tal. Se sugiere restringirse a las composiciones farmacéuticas sustentadas en la descripción. Igualmente el término adhistaminas no es reconocido en el campo técnico. Se requiere definirlo correctamente. Además utiliza el término "al menos" el cual no es limitativo ni preciso y no permite distinguir la invención del estado de la técnica. Se sugiere sustituirlo por un término preciso o un rango concreto. Igual observación aplica para las reivindicaciones 18 y 34.

Las reivindicaciones 4 y 5 no están sustentadas en la descripción puesto que al revisar en la descripción se observa en los ejemplos que el segundo agente activo se selecciona entre bromhidrato de dextrometorfano, guaifenesina, acetaminofen y maleato de clorfeniramina. Se sugiere restringirse a las composiciones farmacéuticas sustentadas en la descripción. Igual observación aplica para las reivindicaciones 19, 20, 35 y 36.

La reivindicación 6 no es clara cuando reclama una composición de una cualquiera de las reivindicaciones 1 a 5 que comprende adicionalmente un

sistema de sabor. Esta manera de reclamar dicho componente no es válida puesto que no define un componente como tal. Se sugiere restringirse a las composiciones farmacéuticas sustentadas en la descripción. Igual observación aplica para la reivindicación 16.

La reivindicación 9 no es clara cuando reclama una composición de una cualquiera de las reivindicaciones 1 a 8 que comprende adicionalmente un antioxidante. Esta manera de reclamar dicho componente no es válida puesto que no define un componente como tal. Se sugiere restringirse a las composiciones farmacéuticas sustentadas en la descripción. Igual observación aplica para la reivindicación 22.

La reivindicación 12 no es clara cuando reclama un endulzante sintético como un componente de la composición. Esta manera de reclamar dicho componente no es válida puesto que no define un componente como tal. Se sugiere restringirse a los componentes utilizados en la composición. Igualmente se utilizan los términos "sustancialmente", "al menos" y "aproximadamente" los cuales no son limitativos ni precisos y no permiten distinguir la invención del estado de la técnica. Se sugiere sustituirlos por términos precisos o rangos concretos.

La reivindicación 14 no está sustentada en la descripción puesto que al revisar en la descripción se observa en los ejemplos que el endulzante sintético corresponde a sucralosa. Se sugiere restringirse a las composiciones farmacéuticas sustentadas en la descripción. Igual observación aplica para la reivindicación 32.

La reivindicación 24 no es clara cuando reclama una composición de una cualquiera de las reivindicaciones 12 a 23 que comprende adicionalmente un agente amortiguador. Esta manera de reclamar dicho componente no es válida puesto que no define un componente como tal. Se sugiere restringirse a las composiciones farmacéuticas sustentadas en la descripción.

Las reivindicaciones 24 y 25 se refieren al pH de la composición el cual es un resultado a alcanzar. Por este motivo no definen al compuesto porque la invención no queda definida por el resultado a alcanzar, puesto que las reivindicaciones incluirían no sólo la solución propuesta por el solicitante, sino todas las alternativas presentes o futuras que lleguen a ese resultado por lo cual no definen las características técnicas esenciales de la invención. Se sugiere eliminar estas reivindicaciones del pliego reivindicatorio.

La reivindicación 27 no es clara cuando reclama una composición de una cualquiera de las reivindicaciones 12 a 26 que comprende adicionalmente un preservativo. Esta manera de reclamar dicho componente no es válida puesto que no define un componente como tal. Se sugiere restringirse a las composiciones farmacéuticas sustentadas en la descripción.

La reivindicación 29 no es clara cuando reclama un endulzante sintético y un agente modificador de viscosidad como componentes de la composición. Esta manera de reclamar dichos componentes no es válida puesto que no define un componente

como tal. Se sugiere restringirse a los componentes utilizados en la composición. Igualmente se utilizan los términos "sustancialmente", "al menos" y "aproximadamente" los cuales no son limitativos ni precisos y no permiten distinguir la invención del estado de la técnica. Se sugiere sustituirlos por términos precisos o rangos concretos.

La reivindicación 31 no es clara puesto que menciona el término "galaotomannonas" como componente de la composición. Este término no es reconocido en el campo técnico por lo cual se requiere definirlo correctamente.

## **6. Análisis de la Oposición**

### **Argumentaciones de Procaps**

Afirma el oponente que esta solicitud pretende obtener patente para el uso de composiciones farmacéuticas ya comprendidas en estado de la técnica por lo cual anexa el documento: WO03047502 publicado el 12 de junio de 2003. Sostiene el solicitante que el documento WO03047502 revela una composición líquida que comprende fenilefrina en la cual se enmascara su sabor desagradable con edulcorantes y agentes viscosantes con lo cual fácilmente una persona versada en la materia llegaría a la conformación de una composición farmacéutica líquida comprendiendo fenilefrina y polietilenglicol sustancialmente libre de aldehído. Motivo por el cual argumenta que la solicitud carece de nivel inventivo en vista de este documento.

### **Respuestas del Solicitante**

Responde el solicitante que la invención revela una composición farmacéutica líquida oral que comprende fenilefrina o una sal farmacéuticamente aceptable de la misma y un polietilenglicol sustancialmente libre de aldehído. El polietilenglicol sustancialmente libre de aldehído tiene menos de 20 ppm de contenido de aldehído total y mantiene dicho nivel de contenido de aldehído durante al menos seis meses. Afirma que el documento presentado menciona la fenilefrina solo como parte de una lista muy general de fármacos con sabor poco placentero y que no hace ninguna referencia a una composición de fenilefrina junto con polietilenglicol sustancialmente libre de aldehído. Motivo por el cual señala que el documento citado por el opositor no afecta la patentabilidad de la solicitud.

### **Respuesta del Examinador Técnico**

El documento WO03047502 divulga una composición farmacéutica líquida que comprende un fármaco de sabor desagradable y polietilenglicol (página 29 reivindicación 1). En la reivindicación 19 (página 31) da a conocer que dicho fármaco de sabor desagradable puede corresponder a un fármaco descongestionante, uno de los cuales puede corresponder a fenilefrina. Sin embargo no divulga que el polietilenglicol utilizado contenga menos de 20 ppm de aldehído. El documento afecta la patentabilidad de la solicitud junto con otros documentos encontrados que divulgan la necesidad de utilizar polietilenglicol con bajo contenido de aldehído para evitar la degradación de los fármacos; dado que divulga la combinación de fenilefrina

y polietilenglicol en una composición farmacéutica que enmascara el sabor desagradable de la fenilefrina. Por lo tanto el documento será tenido en cuenta en el examen de patentabilidad.

7. Determinación del Estado de la Técnica

El Estado de la Técnica se determinó con base en la fecha de Presentación de la Solicitud de Prioridad: Julio 14 de 2006, y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

| Item | Documento  | Título                                                 | Fecha de Publicación |
|------|------------|--------------------------------------------------------|----------------------|
| D1   | WO03047502 | Taste Masked Aqueous Liquid Pharmaceutical Composition | 12/Junio/2003        |
| D2   | US6187340  | Stabilized Pharmaceutical Preparation                  | 13/Febrero/2001      |

D1  
<http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=WO&NR=03047502A1&KC=A1&FT=D&ND=3&date=20030612&DB=worldwide.espacenet.com&locale=en> EP divulga (página 3 línea 1 a página 4 línea 26, página 29 reivindicación 1, página 31 reivindicación 19) una composición de fenilefrina y polietilenglicol

D2  
<http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=US&NR=6187340B1&KC=B1&FT=D&ND=3&date=20010213&DB=worldwide.espacenet.com&locale=en> EP divulga (columna 1 líneas 56 a 65, columna 6 líneas 44 a 49, columna 8 líneas 30 y 31) una composición farmacéutica estabilizada para evitar la degradación del fármaco debido al contenido de aldehído del polietilenglicol

8. Examen de Patentabilidad (Art 14 D486)

Nivel Inventivo (Art18 D486)

La reivindicación 1 consiste en una composición farmacéutica líquida oral que comprende:

- a) Fenilefrina o una sal farmacéuticamente aceptable de esta; y
- b) polietilenglicol sustancialmente libre de aldehídos, en donde el polietilenglicol sustancialmente libre de aldehídos tiene menos de 20ppm de contenido total de aldehído y mantiene dicho nivel de contenido de aldehído durante al menos seis meses.

Comparación de las características técnicas esenciales, con las del Estado de la Técnica

| SOLICITUD                                                 | D1                                             | D2                                     |
|-----------------------------------------------------------|------------------------------------------------|----------------------------------------|
| Composición farmacéutica líquida oral que comprende       | Composición farmacéutica líquida que comprende | Composición farmacéutica estabilizada  |
| Fenilefrina o una sal farmacéuticamente aceptable de esta | Fenilefrina (página 31, línea 19)              | Fenilefrina (columna 8 líneas 30 y 31) |

|                                                                                                                                                                                                                                                       |                                         |                                                                  |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------|------------------------------------------------------------------|
| Polietilenglicol sustancialmente libre de aldehídos, en donde el polietilenglicol sustancialmente libre de aldehídos tiene menos de 20ppm de contenido total de aldehído y mantiene dicho nivel de contenido de aldehído durante al menos seis meses. | Polietilenglicol<br>(Página 29 línea 1) | Polietilenglicol<br>(columna 9 líneas 60 y 61, reivindicación 7) |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------|------------------------------------------------------------------|

El documento D1 divulga una composición que contiene fenilefrina y polietilenglicol (página 3 línea 1 a página 4 línea 26, página 29 reivindicación 1, página 31 reivindicación 19).

La diferencia entre la invención, definida en la reivindicación 1 y la composición de D1 consiste en que D1 no divulga que el polietilenglicol debe contener menos de 20 ppm de aldehído.

El documento D2 divulga la necesidad de estabilizar una composición farmacéutica cuya estabilidad se vea afectada por el contenido de aldehído (columna 1 líneas 56 a 65, columna 6 líneas 44 a 49) donde el principio activo pueda corresponder a fenilefrina (columna 8 líneas 30 y 31)

La diferencia entre la invención, definida en la reivindicación 1 y la composición de D2 consiste en que D2 no divulga que la composición corresponde a una preparación líquida.

El efecto que logra el incluir el uso de polietilenglicol con bajo contenido de aldehído es que previene la degradación de la fenilefrina.

Por lo tanto, el Problema Técnico Objetivo que pretende resolver esta invención se puede formular así: “cómo modificar la composición conocida en D1 para evitar la degradación de la fenilefrina.

Sin embargo, la estabilización de composiciones que se degradan por el contenido de aldehído presente como impurezas en el polietilenglicol ya está divulgada en el documento D2 que se refiere al uso de un estabilizante para inhibir la degradación de un fármaco producida por el contenido de aldehído (columna 1 líneas 56 a columna 2 línea 8, columna 6 líneas 44 a 49)

En consecuencia, la persona del oficio normalmente versada en la materia, utilizaría excipientes libres de aldehído según se divulga su necesidad en D2 en la composición que contiene fenilefrina y polietilenglicol que se divulga en D1. Igualmente polietilenglicol con bajo contenido de aldehído (al igual que las técnicas de detección y cuantificación de aldehídos) ya es conocido en el estado de la técnica<sup>1</sup> y se encuentra disponible comercialmente según indica el solicitante (Ver folios 36 y 37, párrafos 0012 y 0013) para llegar así al objeto de la reivindicación 1 de la solicitud. Por lo cual se considera obvia.

1 Li. Z, Jacobus. L.K, Wuelfing. W.P, Golden. M, Martin. G.P, Reed. R.A "Detection and quantification of low-molecular-weight aldehydes in pharmaceutical excipients by headspace gas chromatography" Journal of Chromatography A, 1104 (2006) 1-10. 10/Enero/2006. (páginas 1 y 2, abstract e introducción, página 8 tabla 2 revela polietilenglicol, contenido de formaldehído de 0.4 ppm. (Ver folios 193 a 202)  
Expediente 09-2495                      1er Art 45

En conclusión la reivindicación 1 cumple el requisito de novedad (Art. 16), pero no cumple con el requisito de nivel inventivo (Art. 18).

Puesto que las reivindicaciones 2 – 10 y 12 – 36 no presentan características inventivas adicionales tampoco se pueden considerar inventivas.

En consecuencia las reivindicaciones 1 – 10 y 12 – 36 cumplen el requisito de novedad (Art. 16), pero no cumplen con el requisito de nivel inventivo (Art. 18).

## 9. Conclusión

Los requisitos de Patentabilidad de la presente Solicitud están afectados así:

| Item | Documento  | Reivindicaciones afectadas | Requisito que afecta |
|------|------------|----------------------------|----------------------|
| D1   | WO03047502 | 1 – 10, 12 – 36            | Nivel Inventivo      |
| D2   | US6187340  | 1 – 10, 12 – 36            | Nivel Inventivo      |

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.

  
**JOSÉ LUIS SALAZAR LÓPEZ**  
 Director de Nuevas Creaciones (C)

El anterior requerimiento fue notificado por fijación en lista, de fecha  
21 AGO. 2012

Elaborado por: Rodolfo Cadena Amaya. *oklt*  
 Revisado por: Gloria Jacqueline Alfonso.



## Detection and quantification of low-molecular-weight aldehydes in pharmaceutical excipients by headspace gas chromatography

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### Abstract

The adverse effect of reactive chemical residues on the quality of drug products has necessitated the determination of low-molecular-weight aldehydes in pharmaceutical excipients. An analytical methodology for the detection of trace amounts of  $C_1$ – $C_8$  aliphatic aldehydes and benzaldehyde in excipients is described. The proposed procedure is based on the derivatization of aldehydes in aqueous solution with *O*-2,3,4,5,6-(pentafluorobenzyl) hydroxylamine hydrochloride (PFBHA), followed by static headspace gas chromatographic (SHS-GC) analysis of PFBHA aldehyde oximes with negative chemical ionization (NCI) MS detection. The method developed was demonstrated to be simple, selective, sensitive, and was successfully applied to the screening of aldehydes at sub- $\mu\text{g/g}$  levels in over 30 typical excipients. The most abundant aldehydes found in the samples were formaldehyde and acetaldehyde, for which a rapid and reliable routine quantification method by readily available SHS-GC instrumentation coupled with flame-ionization detection was also developed and validated.

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**Keywords:** Aldehydes; Excipients; Headspace; GC-MS; Negative chemical ionization; Pentafluorobenzylhydroxylamine hydrochloride

### 1. 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 [1]. There is an increasing awareness of the necessity of a better understanding of 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 [2–4]. These by-products may pose safety questions and, therefore, need to be qualified by toxicological studies that may result in delays or complications of clinical programs. 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 degradate in many drug products [5]. This is particularly true where the reactive chemical entity in the excipient in question has a low-molecular-weight, such as short-chain aldehydes. 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 [6,7]. It has also been reported that formaldehyde can induce crosslinking in gelatin capsules causing an adverse effect on in-vitro dissolution rates of drugs [8,9]. 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. Unfortunately, pharmacopoeial monographs for excipients in major compendia (U.S. Pharmacopoeia-National Formulary, European Pharmacopoeia, Japanese Pharmacopoeia) rarely require the testing for aldehydes, nor are excipient manufacturers willing to provide information on potentially damaging residues in their products for fear that this information could be exploited by a competitor. There have been limited reports of analysis of formaldehyde in excipients and drug products using colorimetric [6] and fluorescent techniques [7]. However, none of these methods has sufficient sensitivity and selectivity

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for the purpose of screening excipients for a broader range of aldehydes. Therefore, the development of a highly sensitive and selective analytical method for the determination of low-molecular-weight aldehydes in pharmaceutical excipients was addressed in our laboratory.

As volatile polar compounds without a strong chromophore, aldehydes are difficult to analyze directly. In the past decades, more sensitive and selective GC and HPLC methods have been developed for aldehydes and ketones after derivatization. There are a large number of classical reagents for the derivatization of aldehydes for chromatographic analyses [10]. Hydrazines (formation of corresponding hydrazones) are the most popular group of derivatizing agents for carbonyl compounds. Several standard HPLC procedures based on the derivatization of aldehydes and ketones with 2,4-dinitrophenylhydrazine (DNPH) have been published [11,12]. However, when employed to analyze low levels of aldehydes in samples of complex matrices these methods have many limitations. Tedious liquid–liquid extractions, which are specified in the procedures to remove derivatization agents and other interferences coming from the sample matrix, may increase risk of error in quantification caused by in-laboratory contamination. *O*-2,3,4,5,6-(Pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA) is an excellent alternative for derivatization of aldehydes (formation of corresponding oximes). The derivatization can be performed under mild reaction conditions (at ambient temperature and in aqueous solution) [13]. The PFBHA-oxime derivatives, which are extremely volatile due to their high fluorine atom content, are amenable to GC analysis and detection by electron capture detection techniques [14,15], resulting in significant improvement of selectivity and sensitivity. Recently, several studies have shown the potential of direct PFBHA derivatization of carbonyls using solid-phase microextraction (SPME) strategies to eliminate liquid–liquid extractions [16–18]. Moreover, the excellent volatility of the PFBHA-oximes enabled the utilization of headspace sampling technique for determination of carbonyl compounds, which minimizes interference from sample matrices. This technique has been applied to the analysis of formaldehyde, acetaldehyde, propionaldehyde, and butyraldehyde in drinking water [19] and ketones in household products [20].

The literature is void of the analysis of aldehydes other than formaldehyde in pharmaceutical excipients and drug products [6,7]. The work presented here describes the development of a systematic approach for the detection and quantification of low-molecular-weight aldehydes in excipients to provide additional insight to formulation development. Specifically, a static headspace GC (SHS-GC) method based on PFBHA derivatization and negative ion chemical ionization (NCI) mass spectrometry with selected-ion monitoring has been developed and employed to screen excipients for the presence of  $C_1$ – $C_8$  aliphatic aldehydes and benzaldehyde. This procedure is highly sensitive and selective for the analytes of interest, and was applied to analyze the aldehyde content in a wide range of excipients. The new method enables a profiling of a spectrum of aldehydes in commonly used pharmaceutical excipients. Additionally, on the basis of SHS-GC-MS screening results, a simple procedure based on the analysis of aldehyde-PFBHA-oximes

by SHS-GC coupled with flame-ionization detection (FID) was developed and validated for the rapid and reliable routine quantification of formaldehyde and acetaldehyde, which were identified as most likely to be present in the excipient samples.

## 2. Experimental

### 2.1. Chemicals

Stock aldehyde standards (certified reference material) (1000  $\mu\text{g/mL}$  of formaldehyde, acetaldehyde, propanal, butanal, pentanal, hexanal, heptanal, octanal, and benzaldehyde in 9:1 acetonitrile/water) were purchased from UltraScientific (North Kingstown, RI, USA). *O*-2,3,4,5,6-(Pentafluorobenzyl)hydroxylamine hydrochloride and 2,4-dinitrophenylhydrazine (DNPH, 97%, moist solid containing  $\sim 30\%$  water) were purchased from Aldrich (Milwaukee, WI, USA). The 1 mg/mL aqueous solution of PFBHA was prepared daily. ACS grade NaCl, 1.0 M HCl solution, HPLC grade acetonitrile and water were purchased from Fisher Scientific (Fair Lawn, NJ, USA). All excipients used were pharmaceutical grade.

### 2.2. Instrumentation

The SHS-GC-MS study was performed with a Thermo Finnigan Trace GC-PolarisQ system (Thermo Finnigan, San Jose, CA, USA) equipped with a capillary split/splitless inlet and COMBI-PAL Liquid and Headspace GC Injection System (Leap Technologies, Carrboro, NC, USA). Chromatographic data were collected and handled via Thermo Finnigan Xcalibur Data System Software (Thermo Finnigan).

The SHS-GC-FID study was performed with a Hewlett-Packard (Agilent, Wilmington, DE, USA) Model 6890 series gas chromatograph (total electronic pneumatic control of gas flow) equipped with a volatiles interface, an HP 7694 headspace autosampler, and an FID. The headspace transfer line was directly connected to the volatiles interface. An auxiliary EPC (Electronic Pressure Control) module was used for vial pressurization. Chromatographic data were collected and handled via the Atlas Chromatography Data System (Thermo Electron, Waltham, MA, USA).

The HPLC analysis was performed with a Hewlett-Packard (Agilent) Model 1100 series liquid chromatograph equipped with a variable wavelength UV detector and an autosampler. Chromatographic data were collected and handled via the Atlas Chromatography Data System (Thermo Electron).

### 2.3. SHS-GC-MS instrumental conditions

The SHS sampling was performed with the COMBI-PAL headspace sampler. A 2.5 mL gastight syringe with a 26 gauge pt no. 5 needle was used (Microliter Analytical Supplies, Suwanee, GA, USA). Carryover in the gas-tight syringe was eliminated by an automatic syringe flush performed after each injection. The headspace autosampler conditions were set as follows: incubation temperature, 80  $^{\circ}\text{C}$ ; incubation time, 20 min; syringe temperature, 105  $^{\circ}\text{C}$ ; agitation speed, 500 rpm; syringe injection

volume, 2.0 mL; syringe fill speed, 0.5 mL/s; syringe injection speed, 0.5 mL/s.

A 30 m  $\times$  0.32 mm I.D. Rtx-5MS column with 1.0  $\mu$ m film thickness (Restek, Bellefonte, PA, USA) was utilized for gas chromatographic separation of the PFBHA aldehyde oximes. The carrier gas was helium at a constant flow rate of 2.0 mL/min. The capillary split/splitless inlet was maintained at 140 °C with a split ratio of 1:10. The column oven temperature program involved an initial temperature of 80 °C for 0 min; increased at 5 °C/min to 220 °C and held for 0 min.

MS scanning experiments were conducted using a solvent delay of 4.0 min and a mass range of 50 to 350 *m/z* with a scan time of 0.50 s/scan. The CI ion source was operated at electron energy of 70 eV. Methane (99%) was used as the reagent gas. All other mass spectrometer conditions were set as follows: methane flow rate, 1.5 mL/min; ion source temperature, 200 °C; transfer line temperature, 250 °C. The ion trap was calibrated using the autotune routine of the PolarisQ software.

#### 2.4. SHS-GC-FID instrumental conditions

The SHS sampling was performed with the HP 7694 headspace sampler. A 1-mL sample loop was employed. The headspace autosampler conditions were set as follows: oven temperature, 45 °C; transfer line temperature, 85 °C; loop temperature, 85 °C; vial equilibration time, 30 min; shaking (mixing) speed, high; loop fill time, 0.15 min; injection (or vent) time, 0.3 min; vial pressure, 10 psi; pressurization time, 0.1 min; loop equilibration time, 0.05 min.

A 60 m  $\times$  0.53 mm I.D. Rtx-1701 column with 1.0  $\mu$ m film thickness (Restek) was utilized for gas chromatographic separation of the PFBHA aldehyde oximes. The carrier gas was helium at a constant flow rate of 10.0 mL/min. The volatiles interface was maintained at 180 °C with a split ratio of 1:5. The FID was set at 250 °C and nitrogen was used as the make-up gas. The column oven temperature program was isothermal 180 °C for 5 min.

#### 2.5. HPLC instrumental conditions

An Inertsil ODS (250 mm  $\times$  4.6 mm, 5  $\mu$ m particle size) HPLC column (GL Sciences, Torrance, CA, USA) was utilized for liquid chromatographic separation of the DNPH derivatives of aldehydes. The mobile phase and sample diluent consisted of 65:35 (v/v) acetonitrile:water. The column temperature was set at 40 °C. Detection of derivatized aldehydes was achieved by UV absorbance at 365 nm with an injection volume of 100  $\mu$ L. The gradient program was isocratic at 1.0 mL/min for 30 min.

#### 2.6. Standard and sample preparation for SHS-GC-MS analysis

A working standard containing 5  $\mu$ g/L of each aldehyde was prepared by dilution of the stock aldehyde standard with water. Headspace standard solutions were prepared by transferring 10.0 mL of working standard and 0.5 mL of 1 mg/mL PFBHA solution into a 20 mL headspace vial and immediately

scaled with a PTFE-lined septum and a stainless steel crimp cap (Microliter Analytical Supplies). A series of standard solutions for method evaluation was prepared by diluting the stock standard with water. Headspace sample solutions were prepared by transferring 100 mg of sample, 10.0 mL of water and 0.5 mL of 1 mg/mL PFBHA solution into a 20 mL headspace vial and immediately sealed with a PTFE-lined septum and a stainless steel crimp cap. (For water insoluble excipients, sample solutions were prepared by transferring ~500 mg of sample into a 50 mL volumetric flask, diluted to volume with water, sealed with parafilm, and stirred for 4 h to enable extraction of aldehydes present. The solution was allowed to settle, and 10.0 mL of the resultant supernatant was transferred into a 20 mL headspace vial along with 0.5 mL of 1 mg/mL PFBHA solution, and immediately sealed with a PTFE-lined septum and a stainless steel crimp cap.)

#### 2.7. Standard and sample preparation for SHS-GC-FID analysis

A working standard containing 10  $\mu$ g/L of each aldehyde was prepared by dilution of the stock aldehyde standard with water. Headspace standard solutions were prepared by transferring 10.0 mL of working standard, 0.5 mL of 1 mg/mL PFBHA solution, and 2 g of NaCl into a 20 mL headspace vial and immediately sealed with a PTFE-lined septum and an aluminum crimp cap (Agilent). A series of standard solutions for method evaluation was prepared by diluting the stock aldehyde standard with water. Quantification was performed by the method of external standardization. Headspace sample solutions were prepared by transferring 100 mg of sample, 10.0 mL of water, 0.5 mL of 1 mg/mL PFBHA solution, and 2 g of NaCl into a 20 mL headspace vial and immediately sealed with a PTFE-lined septum and an aluminum crimp cap. (For water-insoluble excipients, sample solutions were prepared by transferring ~500 mg of sample into a 50 mL volumetric flask, diluted to volume with water, sealed with parafilm, and stirred for 4 h to enable extraction of aldehydes present. The solution was allowed to settle, and 10.0 mL of the resultant supernatant was transferred into a 20 mL headspace vial along with 0.5 mL of 1 mg/mL PFBHA solution and 2 g of NaCl, and immediately sealed with a PTFE-lined septum and a stainless steel crimp cap.)

#### 2.8. Standard and sample preparation for HPLC analysis

An aldehyde standard solution containing 10.0  $\mu$ g/mL of each aldehyde was prepared by dilution of the stock aldehyde standard with HPLC sample diluent (65:35 acetonitrile:water). A working standard solution was prepared by transferring 1.0 mL of the 10.0  $\mu$ g/mL aldehyde standard, 2.0 mL of 0.35 mg/mL DNPH in sample diluent, and 1.0 mL of 1 M HCl to a 10 mL volumetric flask, shaking vigorously for 20 min for derivatization, then diluting to volume with diluent. Sample solutions were prepared by transferring ~1 g of sample into a scintillation vial, adding 10 mL of diluent, and shaking vigorously for 15 min to enable extraction of aldehydes present. The solution was then transferred into a centrifuge tube, and cen-

trifuged for 5 min at 4000 rpm or until the solution was clear. 5.0 mL of the resultant supernatant was transferred into a 10 mL volumetric flask along with 2.0 mL of 0.35 mg/mL DNPH in sample diluent and 1.0 mL of 1 M HCl. The flask was then shaken vigorously for 20 min for derivatization and diluted to volume with diluent.

### 3. Results and discussion

#### 3.1. Preliminary investigation

Prior to the selection of an analytical technique for the determination of low-molecular-weight aldehydes ( $C_1$ – $C_8$  aliphatic aldehydes and benzaldehyde) 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 [1]. For a small molecule API with a molecular weight of 500, the presence of aldehydes at  $\sim 1 \mu\text{g/g}$  in excipients may result in a level of formaldehyde adduct as high as 0.2%, the lowest qualification threshold specified in the ICH guideline [5]. Therefore,  $1 \mu\text{g/g}$  was thought to be reasonable as a threshold for the total content of low-molecular-weight aldehydes in excipients.

The applicability of HPLC techniques based on derivatization of aldehydes with DNPH was first investigated. A multi-step procedure adopted from EPA method 8315A [12] was applied to excipient samples (Section 2.8). It was found that the method did not provide sufficient sensitivity and/or selectivity required for this study. Typical HPLC chromatograms obtained from excipient samples are shown in Fig. 1. Interference from the derivatizing agents and/or sample matrices was observed. These artifacts made it very difficult to unambiguously identify and to quantitate the sub- $\mu\text{g/g}$  levels of aldehydes in the samples. Additionally, it was noticed that many excipients form gelatinous solutions, which were extremely difficult to clarify in order to obtain an injectable sample solution for HPLC analysis. Hence, the HPLC method was considered to be unsuitable as a general procedure for the determination of aldehydes in excipients.

#### 3.2. Development of SHS-GC-MS method for aldehyde screening

An alternative approach of derivatizing aldehydes with PFBHA was then considered for the task. The PFBHA derivatization chemistry is well established and provides a simple one-step aqueous sample preparation. The absence of organic reagents/solvents other than the derivatizing agent PFBHA eliminates potential cross contamination. The high volatility of fluorine-rich PFBHA-oxime derivatives enables the use of capillary gas chromatography, which offers excellent selectivity, and opens the door to static headspace (SHS) sampling, which minimizes the interference from complex sample matrices.

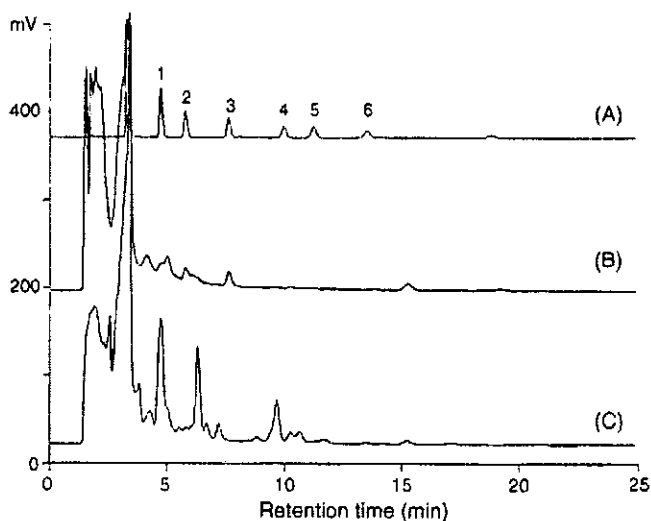


Fig. 1. HPLC chromatograms for analysis of aldehydes in excipients after derivatization with DNPH. Method conditions are shown in Section 2.5. (A) Method standard containing 1.0 mg/mL levels of  $C_1$ – $C_6$  aldehydes. (B) A sample of hydroxypropyl cellulose. (C) A sample of hypromellose. Peaks: 1 = formaldehyde, 2 = acetaldehyde, 3 = propanal, 4 = butanal, 5 = pentanal, 6 = hexanal.

The initial objective of the study was to screen pharmaceutical excipients for the presence of  $C_1$ – $C_8$  aliphatic aldehydes and benzaldehyde. Therefore, an SHS-GC procedure with mass spectrometric (MS) detection was highly desirable for both the added sensitivity and to readily identify any of these aldehydes present in excipient samples. Experimental conditions that could impact method selectivity and sensitivity during chromatographic analysis, sample preparation and derivatization, and headspace sampling were carefully examined when developing the SHS-GC-MS method.

#### 3.2.1. Chromatographic conditions

**3.2.1.1. Selectivity.** The selectivity of the method was established using a mixture of PFBHA-oxime derivatives of aldehydes, prepared by derivatizing a standard mixture of  $C_1$ – $C_8$  aliphatic aldehydes and benzaldehyde with PFBHA. The resultant derivatized sample was then chromatographed by capillary GC coupled with an ion trap mass spectrometer for peak detection. A low bleeding 5%-phenyl siloxane column specially designed for MS (Rtx-5MS) was employed for initial studies. Negative ion chemical ionization MS detection was utilized (with methane as the reagent gas) because much better signal intensity and characteristic peaks for each of aldehydes can be achieved in NCI mode, attributed to the presence of the pentafluorobenzyl group in the aldehyde-oxime derivatives [19,20]. The obtained SHS-GC-MS NCI mass chromatogram of PFBHA-oximes of  $C_1$ – $C_8$  aldehydes and benzaldehyde is shown in Fig. 2. Although the sample involved starting with ten components, PFBHA and nine aldehydes, a total of 17 peaks are observed. It is useful to note that all aldehyde derivatives, except formaldehyde-PFBHA-oxime, can form *syn* (I) and *anti* (II) stereoisomers of the oximes (see Fig. 3) since the carbon–nitrogen double (imine) bond prevents both rotation and ammonia-like inversion. Each peak was then identified by either injection of a

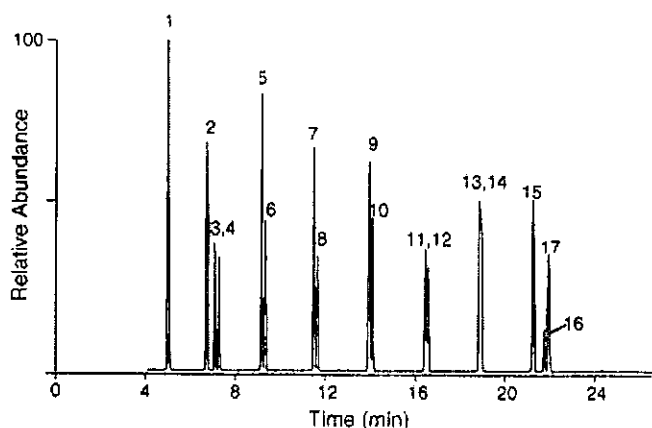


Fig. 2. Typical SHS-GC-MS NCI total ion count mass chromatogram of PFBHA aldehyde oximes. Method conditions are shown in Section 2.3. Peaks: 1 = formaldehyde-PFBHA oxime; 2 = PFBHA; 3, 4 = acetaldehyde-PFBHA oximes; 5, 6 = propanal-PFBHA oximes; 7, 8 = butanal-PFBHA oximes; 9, 10 = pentanal-PFBHA oximes; 11, 12 = hexanal-PFBHA oximes; 13, 14 = heptanal-PFBHA oximes; 15 = octanal-PFBHA oximes; 16, 17 = benzaldehyde-PFBHA oximes.

PFBHA-reacted individual component, or PFBHA itself. Peak 2 in the chromatogram (Fig. 2) is then readily attributed to unreacted PFBHA. As expected, formaldehyde only forms one peak (Peak 1 in Fig. 2). The two isomers of  $C_2$ – $C_5$  aldehydes and benzaldehyde derivatives are resolved by GC while only partial separation was achieved for  $C_6$  and  $C_7$  derivatives and no separation obtained for octanal derivatives.

**3.2.1.2. Identification.** To further confirm the identity of the derivatives, NCI mass spectra of each peak in full scan were acquired and analyzed for MS fragmentation characteristics. The NCI mass spectra of the PFBHA-oximes of formaldehyde, pentanal, and benzaldehyde derivatives are shown in Fig. 4(A)–(C), respectively, and the results are summarized in Table 1. The MS spectra for other aliphatic aldehydes (not shown) are similar to that of pentanal. Both the base peak at  $m/z$  181 and the peak at

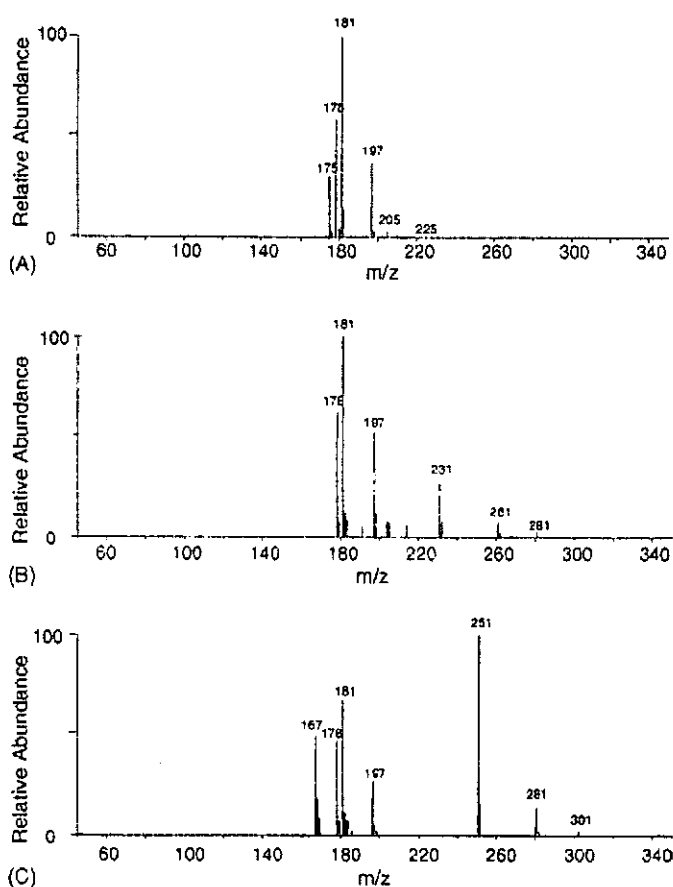


Fig. 4. Negative ion chemical ionization mass spectra of PFBHA aldehyde oximes. Method conditions are shown in Section 2.3. (A) Formaldehyde-PFBHA oxime. (B) Pentanal-PFBHA oximes. (C) Benzaldehyde-PFBHA oximes.

$m/z$  197 observed for  $C_1$ – $C_8$  and benzaldehyde derivatives result from the PFBHA derivatizing group, and are due to the pentafluorobenzyl anion  $C_6F_5CH_2^-$  and pentafluorobenzoyloxy anion  $C_6F_5CH_2O^-$ , respectively. Additionally, the derivatized molecular ions ( $M^-$ ) lose HF to produce  $(M-HF)^{-\bullet}$  [or  $(M-20)^{-\bullet}$ ]

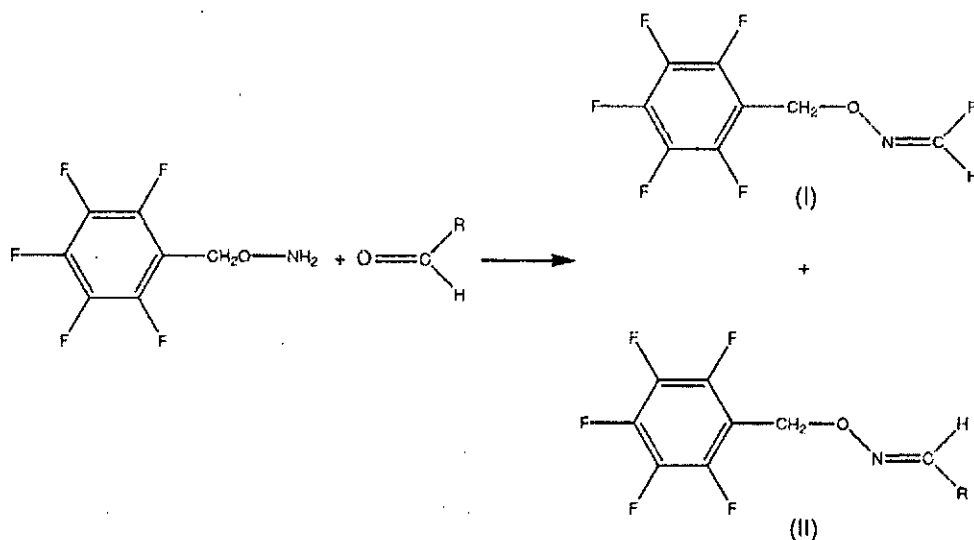


Fig. 3. Derivatization reaction of aldehydes with the reagent PFBHA.

Table 1  
Peak identification and figures of merit for the SHS-GC-MS procedure

| Aldehydes    | Peak no. (Fig. 2) | Characteristic ions of NCI mass spectra |                       |                       |                         | Linearity    |                | DL <sup>a</sup> |                      | RSD <sup>b</sup> (%) |
|--------------|-------------------|-----------------------------------------|-----------------------|-----------------------|-------------------------|--------------|----------------|-----------------|----------------------|----------------------|
|              |                   | (M) <sup>+</sup> •                      | (M-20) <sup>+</sup> • | (M-50) <sup>+</sup> • | PFBHA ions <sup>c</sup> | Range (µg/L) | R <sup>2</sup> | (µg/L)          | (µg/g <sup>d</sup> ) |                      |
| Formaldehyde | 1                 | 235                                     | 205                   | 175                   | 178, 181, 197           | 0.5–20       | 0.9957         | 0.5             | 0.05                 | 3.1                  |
| Acetaldehyde | 3, 4              | 239                                     | 219                   | 189                   | 178, 181, 197           | 1–20         | 0.9950         | 1.0             | 0.1                  | 3.2                  |
| Propanal     | 5, 6              | 253                                     | 233                   | 203                   | 178, 181, 197           | 0.5–20       | 0.9977         | 0.2             | 0.02                 | 2.0                  |
| Butanal      | 7, 8              | 267                                     | 247                   | 217                   | 178, 181, 197           | 0.5–20       | 0.9957         | 0.2             | 0.02                 | 3.0                  |
| Pentanal     | 9, 10             | 281                                     | 261                   | 231                   | 178, 181, 197           | 0.5–20       | 0.9947         | 0.2             | 0.02                 | 4.6                  |
| Hexanal      | 11, 12            | 295                                     | 275                   | 245                   | 178, 181, 197           | 0.5–20       | 0.9952         | 0.5             | 0.05                 | 4.7                  |
| Heptanal     | 13, 14            | 309                                     | 289                   | 259                   | 178, 181, 197           | 0.5–20       | 0.9977         | 0.5             | 0.05                 | 3.5                  |
| Octanal      | 15                | 323                                     | 303                   | 273                   | 178, 181, 197           | 0.5–20       | 0.9987         | 0.5             | 0.05                 | 2.8                  |
| Benzaldehyde | 16, 17            | 301                                     | 281                   | 251 <sup>c</sup>      | 178, 181, 197           | 1–20         | 0.9901         | 1               | 0.1                  | 5.6                  |

<sup>a</sup> DL = 3.3 × (standard deviation of blank)/(slope of calibration curve) [23].  
<sup>b</sup> Pooled RSD (*n* = 20) at five concentration levels over the linearity range.  
<sup>c</sup> Ions selected for quantitation.  
<sup>d</sup> Concentration expressed on a weight basis relative to a 10 mg/mL sample concentration = {(DL) (µg/L)/10 (mg/mL)}.

ions, which give more intense signals than do the corresponding molecular ions in the mass spectra. The (M-HF)<sup>+</sup>• ions undergo further dissociation to produce ions *m/z* 178 and (M-HF-30)<sup>+</sup>• [or (M-50)<sup>+</sup>•] which also present at even higher abundance in the mass spectra. In contrast, the base peak for benzaldehyde derivatives is shown as the (M-50)<sup>+</sup>• ion of *m/z* 251. Other ions observed for benzaldehyde include *m/z* 281 (M-30)<sup>+</sup>•, *m/z* 167 (C<sub>6</sub>F<sub>5</sub><sup>+</sup> anion), *m/z* 178, *m/z* 181, and *m/z* 197. The parent ion was observed for all peaks, thus confirming the stated molecular identity. Furthermore, *syn*- and *anti*-pairs exhibited identical mass spectral features, supporting the presence of both components for the C<sub>2</sub>–C<sub>7</sub> and benzaldehyde derivatives. These MS ion peaks along with chromatographic elution time were used as the definitive identification of the aldehydes detected in the excipient samples.

**3.2.1.3. Sensitivity:** The unique characteristics of the NCI spectra of aldehyde PFBHA-oxime derivatives not only provide excellent selectivity, but they also afford excellent method sensitivity. Since all the primary fragments arising from the derivatizing group (*m/z* 178, *m/z* 181, and *m/z* 197) were observed for all aldehyde PFBHA derivatives, the detection of all analytes was run in the selected-ion monitoring (SIM) mode with monitoring for these main MS fragments (Table 1) to maximize the sensitivity of the method. (M-50)<sup>+</sup>• ion of *m/z* 251 was also selected for benzaldehyde derivative. The final chromatographic conditions are given in Section 2.3.

**3.2.2. Optimization of sample preparation and headspace sampling**

Sample preparation solvent composition and sample concentration are two critical factors for sensitivity and accuracy in headspace analysis [21]. One of the primary advantages of PFBHA derivatization is that it can be done in aqueous solution, desirable for headspace analysis as water provides generally very clean backgrounds. Furthermore, it was possible to employ a much smaller sample size (5–10 mg/mL) in this study than the HPLC-DNPH method (100 mg/mL), thus minimizing sample matrix effects, due to the excellent sensitivity of NCI-SIM

detection. Dilute solutions also favor more complete extraction of aldehydes particularly from excipients that may have limited solubility in water. Insoluble excipients were subjected to a 4-h extraction in water prior to derivatization (see Sections 2.6 and 2.7) to ensure complete extraction of aldehydes present. The derivatization of aldehydes with PFBHA proceeds readily in weakly acidic to neutral media [13] that encompasses all excipient sample solutions studied, making the use of a buffer solution unnecessary. A number of authors have also noted that the condensation reaction was complete in water in less than 10 min at room temperature [13,18,19]. Therefore, the formation of the oximes was assumed to occur in less 10 min and thus within the headspace sample solution equilibrium discussed below.

There are many instrumental parameters of a headspace autosampler that can affect the performance of static headspace analysis. Parameters other than headspace vial equilibration (or incubation) temperature and time are normally not analyte specific. Therefore, settings for these parameters, which had been optimized for analysis of organic volatile impurities in excipients [22], were adopted in this study. The equilibration temperature was examined across a temperature range (25–80 °C) and set at 80 °C to ensure adequate sensitivity was observed for the later eluting C<sub>5</sub>–C<sub>8</sub> aldehydes and benzaldehyde. The equilibrium in the headspace vial was reached in 20 min at 80 °C for all analytes. The final SHS-GC-MS method conditions are given in Sections 2.3 and 2.6.

**3.2.3. Method evaluation**

The linearity of the method was evaluated from a series of standard solutions prepared for each aldehyde over the concentration range listed in Table 1. Results for linearity are summarized in the table along with method precision data. All relative standard deviations (RSDs) observed are equal to or less than 5.6%. Each analyte showed excellent linear behavior over the examined concentration range with coefficient of determination (*R*<sup>2</sup>) values of ≥0.99. Determination of the sensitivity of the method was challenged by the presence of low molecular weight aldehydes (in particular, formaldehyde and acetaldehyde) which exist at relative high levels in the water used for this study. A

blank run on the water used for standard and sample preparations followed by subsequent subtraction of blank values from that of each standard/sample run was used to overcome this limitation. Detection limits (DL) were calculated based on the standard deviation ( $\sigma$ ) of the blank and the slope ( $S$ ) of the calibration curve ( $DL = 3.3\sigma/S$ ) [23] and reported in Table 1 as the DLs in solution ( $\mu\text{g/L}$ ) with corresponding DLs in excipient samples ( $\mu\text{g/g}$ ) involving 10 mg/mL sample concentrations. Table 1 shows that all DLs in excipient samples are  $\leq 0.1 \mu\text{g/g}$ , exceeding the desired method sensitivity for the screening of aldehyde reactivity as discussed in Section 3.1.

### 3.3. Screening of aldehydes in excipient samples

The SHS-GC-MS method was applied to screen a wide range of excipients for the presence of low-molecular-weight aldehydes. The facile one-step sample preparation significantly improved the overall sample preparation efficiency. Typical NCI mass chromatograms of excipient samples are shown in Fig. 5, which indicates that the SHS-GC-MS procedure offers significantly improved chromatography compared with HPLC analysis (see Fig. 1). Sample matrix artifacts other than a derivatizing agent peak were not observed. Fig. 5 shows that the 200:1 excess PFBHA used in the derivatization does not interfere with the detection of aldehydes. All detected aldehydes were clearly identified based on unique retention time and characteristic ion peaks for each analyte (Table 1). A total of 31 excipients were analyzed and the results are summarized in Table 2. Among various classes of excipients tested, all inorganic materials, such as NaCl, titanium dioxide, and dibasic calcium phosphate, were found not to contain any aldehydes as expected. Antioxidants (butylated hydroxyanisole and propyl gallate) and lipids (caster oil, corn oil, and medium chain glycerides) were also found to contain negligible amounts of aldehydes. Detectable amounts of  $C_4$ – $C_8$  aldehydes or benzaldehyde were not found in any of the samples examined. Propanal was only found in a sample of poloxamer

407, which is known to start thermal degradation at  $\sim 80^\circ\text{C}$  to form aldehydes [24,25] and can be attributed to the headspace incubation procedure used here (*vide infra*). Formaldehyde and acetaldehyde were observed in many excipients. Low levels of formaldehyde and acetaldehyde ( $\leq 0.2 \mu\text{g/g}$ ) were detected in sugars (lactose, sucrose, and mannitol) and lubricants (magnesium stearate and sodium stearyl fumarate). Moderate amounts of aldehydes (less than  $1 \mu\text{g/g}$ ) were found in microcrystalline cellulose, hydroxypropyl cellulose, croscarmellose sodium, and triethyl citrate. Formaldehyde and/or acetaldehyde were found to be present at relatively high levels ( $>2 \mu\text{g/g}$ ) in ethylcellulose, partially pregelatinized corn starch, polyvinylpyrrolidone (povidone and crospovidone), and various grades of polyethylene glycols (PEGs), and therefore, are potential contributors to degradation of formulations as discussed in Section 3.1. This analytical technique then becomes a vital tool to assess aldehyde content which may lend itself to guide product design considerations.

### 3.4. Rapid quantification of formaldehyde and acetaldehyde

Though highly sensitive and selective, the SHS-GC-MS procedure requires sophisticated GC-MS instrumentation, which may not readily available in a typical analytical laboratory. Therefore, a simple and reliable method for routine determination of aldehydes is highly desirable. Since  $C_3$ – $C_8$  aldehydes and benzaldehyde were not found in most excipients screened by GC-MS, it was decided that the primary objective of the rapid method is to quantify formaldehyde and acetaldehyde. GC with FID was then explored in regard to acceptable sensitivity, and is preferred as it is the standard instrument in most pharmaceutical laboratories.

#### 3.4.1. Method optimization

Although providing great sensitivity for MS detection and generally satisfactory separation for all analytes of interest, the low-bleeding 5%-phenyl siloxane column used in the GC-MS procedure does have a drawback in that the acetaldehyde-oximes eluted after the PFBHA (Fig. 2), which may effect the quantitation of acetaldehyde in a fast chromatographic method. The elution order was reversed on a column coated with a more polar (17%-cyanopropyl-phenyl)methylpolysiloxane stationary phase (such as Rtx-1701), which was then selected for the rapid method. A long (60 m) megabore (0.53 mm I.D.) column was used as it has the highest sample capacity needed for sensitivity while maintaining the superior separation efficiency characteristic of capillary columns at a high carrier gas flow rate (10 mL/min). The fast flow shortens the run time and is also preferred for headspace analysis to reduce peak broadening due to dead volumes in headspace sampler components. The incubation temperature was lowered to  $45^\circ\text{C}$  to minimize the potential degradation of excipients, such as poloxamer 407. Another advantage of using the  $45^\circ\text{C}$  incubation temperature is that interference from less volatile components, which may elute after the PFBHA peak, is minimized and thus allows a very short bake-out time. A salting-out method was used by saturating the headspace

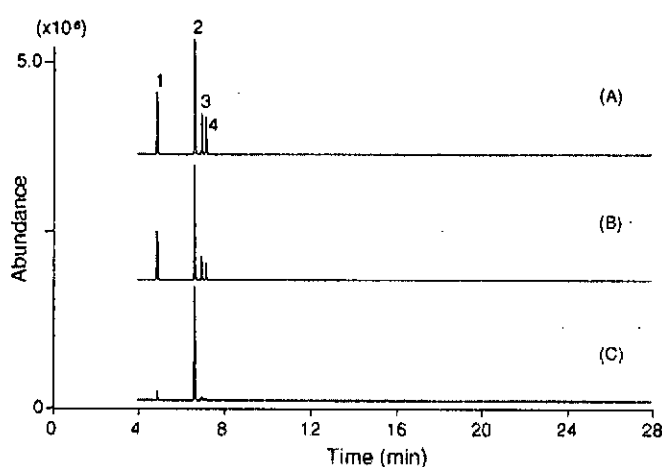


Fig. 5. SHS-GC-MS NCI mass chromatograms for screening aldehydes in excipients. Method conditions are shown in Section 2.3. (A) A sample of hypromellose. (B) A sample of hydroxypropyl cellulose. (C) The PFBHA blank. Peaks: 1 = formaldehyde-PFBHA oxime; 2 = PFBHA; 3, 4 = acetaldehyde-PFBHA oximes.

Table 2  
SHS-GC-MS screening results

| Excipients                           | Aldehydes detected (μg/g) <sup>a</sup> |                  |          |        |
|--------------------------------------|----------------------------------------|------------------|----------|--------|
|                                      | Formaldehyde                           | Acetaldehyde     | Propanal | Others |
| Butylated hydroxyanisole             | N.D.                                   | N.D.             | N.D.     | N.D.   |
| Caster oil                           | N.D.                                   | N.D.             | N.D.     | N.D.   |
| Corn oil                             | N.D.                                   | N.D.             | N.D.     | N.D.   |
| Croscarmellose sodium                | 0.5                                    | 0.3              | N.D.     | N.D.   |
| Crospovidone                         | 2.4                                    | 4.5              | N.D.     | N.D.   |
| Dibasic calcium phosphate            | N.D.                                   | N.D.             | N.D.     | N.D.   |
| Ethylcellulose                       | 2.5                                    | >10 <sup>b</sup> | N.D.     | N.D.   |
| Hydroxypropyl cellulose              | 0.6                                    | 0.5              | N.D.     | N.D.   |
| Hypromellose                         | 1.6                                    | 0.1              | N.D.     | N.D.   |
| Lactose                              | 0.1                                    | N.D.             | N.D.     | N.D.   |
| Magnesium stearate                   | 0.1                                    | N.D.             | N.D.     | N.D.   |
| Mannitol                             | 0.2                                    | N.D.             | N.D.     | N.D.   |
| Medium-chain triglycerides           | N.D.                                   | N.D.             | N.D.     | N.D.   |
| Microcrystalline cellulose           | 0.4                                    | 0.1              | N.D.     | N.D.   |
| Mono- and di-glycerides              | N.D.                                   | N.D.             | N.D.     | N.D.   |
| Partially pregelatinized corn starch | 2.5                                    | 0.1              | N.D.     | N.D.   |
| Poloxamer 407                        | 0.1                                    | 2.0              | 0.9      | N.D.   |
| Polyethylene glycol 400              | >10 <sup>b</sup>                       | 8.2              | N.D.     | N.D.   |
| Polyethylene glycol 600              | >10 <sup>b</sup>                       | 4.1              | N.D.     | N.D.   |
| Polyethylene glycol 1450             | 0.4                                    | 0.7              | N.D.     | N.D.   |
| Polyethylene glycol 4000             | 0.4                                    | 2.1              | N.D.     | N.D.   |
| Polyoxyl 35 castor oil               | 1.1                                    | N.D.             | N.D.     | N.D.   |
| Polysorbate 80                       | 1.6                                    | 0.6              | N.D.     | N.D.   |
| Povidone                             | 4.7                                    | 4.2              | N.D.     | N.D.   |
| Propyl gallate                       | N.D.                                   | N.D.             | N.D.     | N.D.   |
| Sodium chloride                      | N.D.                                   | N.D.             | N.D.     | N.D.   |
| Sodium starch glycolate              | 0.1                                    | 2.7              | N.D.     | N.D.   |
| Sodium stearyl fumarate              | 0.1                                    | N.D.             | N.D.     | N.D.   |
| Sucrose                              | 0.1                                    | N.D.             | N.D.     | N.D.   |
| Titanium dioxide                     | N.D.                                   | N.D.             | N.D.     | N.D.   |
| Triethyl citrate                     | 0.2                                    | 0.6              | N.D.     | N.D.   |

<sup>a</sup> N.D.: not detected.  
<sup>b</sup> Peak signal exceeded the linearity range.

sample solution with NaCl to yield a sensitivity increase of 4–5-fold. Additionally, salting-out reduces sample matrix effects, and therefore improves method accuracy. It also shortens the equilibration time in the headspace vial. The reported effect of increased viscosity of high salt concentration on the incubation time [21] was not observed. It may be due to the fact that a much lower salt concentration was used in this study (17% w/w) compared to that (55% w/w) in the literature. The optimal vial equilibration time was empirically determined to be 30 min. In addition, no interference of potential volatile impurities in the salt was observed. The ACS grade NaCl was used without any pretreatment.

3.4.2. Method evaluation

Representative SHS-GC-FID chromatograms of a derivatization blank, a working standard, and a sample using optimized experimental conditions are illustrated in Fig. 6. All analytes of interest are well resolved from each other. Two isomers of the acetaldehyde derivative are separated and can be used as an additional identification criterion. Since the Agilent 7694 headspace autosampler allows the staggering of the start of vial incubation time, a complete analysis was achieved within 1–2 h, much less than the typical overnight run needed for the

SHS-GC-MS analysis. The performance characteristics of the SHS-GC-FID procedure were evaluated with respect to precision, accuracy, linearity, and range. Both formaldehyde and acetaldehyde showed excellent linear behavior over the concentration range of 5–200 μg/L in solution with coefficient of

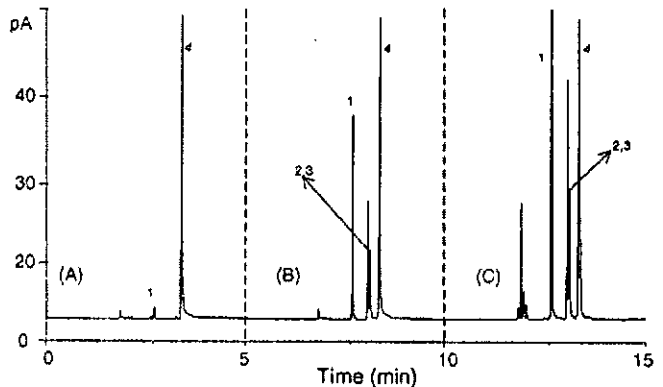


Fig. 6. SHS-GC-FID chromatograms for quantification of aldehydes in excipients. Method conditions are shown in Section 2.4. (A) The PFBHA blank. (B) The working standard (see Section 2.7). (C) A sample of PEG 400. Peaks: 1 = formaldehyde-PFBHA oxime; 2, 3 = acetaldehyde-PFBHA oximes; 4 = PFBHA.

Table 3  
SHS-GC-FID quantification results

| Excipients                           | Aldehydes detected (µg/g) |              |
|--------------------------------------|---------------------------|--------------|
|                                      | Formaldehyde              | Acetaldehyde |
| Croscarmellose sodium                | 0.6                       | 0.2          |
| Crospovidone                         | 3.1                       | 3.9          |
| Ethylcellulose                       | 2.0                       | 15.9         |
| Hydroxypropyl cellulose              | 0.4                       | 0.5          |
| Hypromellose                         | 2.1                       | <0.2         |
| Microcrystalline cellulose           | 0.4                       | <0.2         |
| Partially pregelatinized corn starch | 2.6                       | <0.2         |
| Poloxamer 407                        | <0.2                      | 0.2          |
| Polyethylene glycol 400              | 196                       | 11.4         |
| Polyethylene glycol 600              | 953                       | 4.2          |
| Polyethylene glycol 1450             | 0.4                       | 0.8          |
| Polyethylene glycol 4000             | 0.5                       | 1.9          |
| Polyoxyl 35 castor oil               | 0.8                       | <0.2         |
| Polysorbate 80                       | 1.8                       | 0.7          |
| Povidone                             | 6.0                       | 4.3          |
| Sodium starch glycolate              | <0.2                      | 3.4          |
| Triethyl citrate                     | 0.2                       | 0.6          |

determination ( $R^2$ ) values of 0.9988 and 0.9995, respectively. The detection limit of the method is equivalent to that of GC-MS procedure (Section 3.2.3) as the sensitivity is again limited by the presence of aldehydes in the blanks. The quantitation limit of the method is defined as the lower end of the linear range, 5 µg/L (0.25 µg/g in excipients for a 10 mg/mL sample). Method precision was assessed by evaluating RSDs of replicate sample preparations for each analyte over the linearity range. The accuracy of the method was verified on excipient samples spiked with known amounts of aldehydes. All RSDs observed were equal to or less than 3% and recoveries were within 85–97%, indicating that the method provides excellent precision and accuracy at the sub-µg/g level.

Excipients which had been screened by GC-MS to contain >0.5 µg/g amounts of formaldehyde and/or acetaldehyde (Table 2) were again quantitated using the rapid SHS-GC-FID procedure with the results summarized in Table 3. The lower incubation temperature eliminates degradation of poloxamer 407 evidenced by only a trace amount of acetaldehyde being present. Comparing Tables 2 and 3 also show that aldehyde values obtained by the two procedures were in a good agreement, with the poloxamer 407 exception noted above, further demonstrating that the incubation temperature employed is adequate for both methods. Aldehydes were found to be present at highest levels in polyethylene glycols (PEGs), which have general popularity in formulation design as they can be used to enhance the solubility and bioavailability of poorly soluble compounds in liquid filled or semi-solid filled gelatin capsules. In particular, liquid PEGs (PEG 400 and PEG 600) were shown to contain significant amounts of formaldehyde, 196 and 953 µg/g, respectively.

4. Conclusion

An analytical methodology for the determination of low-molecular-weight aldehydes in pharmaceutical excipients has

been described. The work presented herein has demonstrated that static headspace gas chromatographic analysis of PFBHA derivatives provides an effective means for detection and quantification of aldehydes in excipients. A highly selective and sensitive SHS-GC-MS procedure for screening of C<sub>1</sub>–C<sub>8</sub> aldehydes and benzaldehyde was developed. The use of a negative chemical ionization mass spectrometry procedure allowed the measurement of trace amounts of PFBHA aldehyde oximes in samples. In addition, a simple and reliable SHS-GC-FID procedure using standard equipment for the rapid quantification of formaldehyde and acetaldehyde in excipients was developed and validated. Typical pharmaceutical excipients were analyzed to demonstrate the suitability of the proposed methodology for the qualitative and quantitative analyses of aldehydes. Excipients containing significant amounts of aldehydes, and possibly inducing significant degradation chemistry in pharmaceutical products, were identified. Preliminary results suggest the presence and amount of aldehydes in commercial materials will vary by manufacturer, and should warrant continued comprehensive study using the presented methodology. Additional studies may also be conducted for a better understanding of aldehyde formation and growth in excipients on storage. With the availability of analytical tools presented here, an ultimate goal of this research would be to deconvolute the correlation between the reactivity of an excipient and its aldehyde content. Another potential applications of the procedure presented includes the determination of aldehydes in the headspace of pharmaceutical packages.

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