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Trace level determination of chloroacetyl chloride and degradation products by derivatization gas chromatography

Marie-France Morissette, Larry Wigman, Jerry Tso*

Small Molecule Pharmaceutical Sciences, Genentech, 1 DNA Way, South San Francisco, CA, 94080, United States

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ABSTRACT

A gas chromatographic procedure has been developed for the trace determination of chloroacetyl chloride (CAC) and two of its impurities: methyl chloroacetate (MCA) and chloroacetic acid (CAA). All three compounds are derivatized using piperidine in dichloroethane prior to their analysis via gas chromatography coupled with a flame ionization detection (GC-FID). Recoveries of each compound were assessed in two different pharmaceutical matrices (intermediate and final active pharmaceutical ingredient) and ranged from 75 to 125%. The limit of quantitation has been determined to be 0.10% wt/wt for CAA and 0.03% wt/wt for CAC and MCA. The linearity ranged from 0.03 to 5.00% wt/wt for CAC and MCA and from 0.10 to 5.00% wt/wt for CAA, with correlation coefficients from 0.9995 to 1.0000. Repeatability was evaluated at LOQ and at 5.00% wt/wt and was found to be between 1.4–3.0%.

1. Introduction

Chloroacetyl chloride (CAC) is a reactive compound that is often used as a two-carbon building block reagent given its multifunctional properties. The wide use of CAC in the pharmaceutical industry as an acylating agent in the synthesis of active pharmaceutical ingredient (API) has shown to be a critical reagent [1–3]. Although limited information are available regarding the genotoxicity and the carcinogenicity of CAC, it can be categorized as an alerting structure for genotoxic potential. The potential genotoxicity of CAC allows for a class 3 impurity based on Muller's classification [4]. The class 3 assignment for CAC is due to the presence of the acyl halide that acts as a strong electrophilic agent known to react with DNA.

The high reactivity of CAC allows reaction with residual moisture and solvents present in either the initial reaction mixture or subsequent steps to form potential degradation species. These impurities of CAC are dependent on the solvents present and should be evaluated based on a case to case basis. Two commonly observed impurities of CAC due to the presence of methanol and water are chloroacetic acid (CAA) and methylchloroacetate (MCA). These two compounds are likely to be less potent due to their monofunctionality compared to bifunctional nature of CAC. However, both compounds are alkyl halides and could be categorized as class 3 compounds [4]. The acceptable daily intake for class 3 compound could range between 1.5–120 μg/day depending on treatment duration [5]. For that reason, it is important to have a thorough understanding of the fate of CAC as well as its impurities in the synthesis process and the proper in-process and release controls during manufacturing of the API.

More than three decades ago, The American Conference of Governmental Industrial Hygienists established the CAC threshold limit value (TLV) for a 8 h time weighted average exposure to be 0.05 ppm [6]. Therefore, many methods for sampling in CAC in air have been developed using a liquid impinger to trap the CAC under its ester form [7,8] or solid sorbent like Tenax-GC coated wlth 9-[(*N*-methylamino)methyl]anthracene [9] or silica [10] coupled along with GC-ECD [7,8] HPLC-UV [9] and ion chromatography [10]. More recently, current methods have been developed to monitor CAC using more advanced techniques such as LC–MS/MS or capillary electrophoresis (CE) [11,12]. These instrumentations are more specialized than gas chromatography commonly found in pharmaceutical quality control labs. The LC–MS/MS method by Wijk et Al. attempted to quantitate CAC, but resulted in issues where the compounds were not stable under their method conditions and could not be analyzed. Khan et al., used CE to directly analyze CAC, although based on the sample preparation the analysis would be of the methylchloroacetate due to preparation in methanol. Most analysis of alkyl halides are derivatized prior to gas chromatography [13,14] or ion chromatography [15,16] and in each paper

* Corresponding author.
E-mail address: tso.jerry@gene.com (J. Tso).

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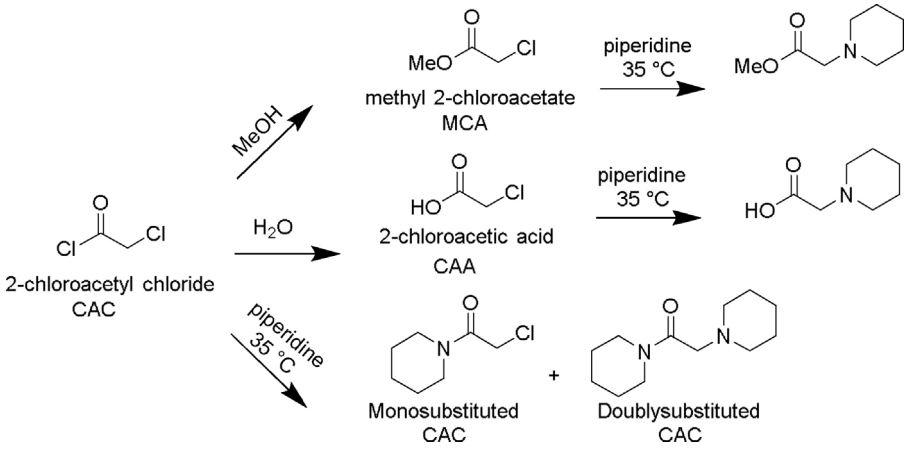

Fig. 1. Chemical structure of chloroacetyl chloride, chloroacetic acid and methyl chloroacetate and their respective derivative.

no CAC impurities were monitored. A related class of compounds are halogenated carboxylic acids that have been targeted in water analysis testing and more recently for pharmaceutical testing due their potential genotoxicity. Hou et al., incorporated a derivatization method using nitro-substituted phenylhydrazines coupled with HPLC analysis for monitoring up to 6 residual analytes in drug substances [17]. The method was significantly improved compared to the traditional analysis of halogenated carboxylic acids in water, where detection of 6 analytes in a single optimized derivatization step had good sensitivity. Even though the method removes the timely pre-treatment steps associated with traditional EPA Methods [18], the optimized conditions used up to 30% water in the sample preparation, leading to potential hydrolysis products that are not targeted in the method. Unlike analysis for CAC, the removal of water is required to prevent the potential impurities forming from hydrolysis. Delinsky et al. was able to avoid all derivatization and pre-treatment steps and retained dichloroacetic acid using hydrophilic interaction liquid chromatography along with mass spectrometry in a more complex matrix of blood and tissue [19]. The elimination of any derivatization step allows for simplification and is ideal routine and quick analysis turnarounds. However, detection by mass spectrometry as previously discussed adds limitations that may not be optimal for routine analysis and the requirement of water in the mobile phase would cause on column degradation of CAC.

In this paper, we describe a simple derivatization technique whereby CAC and its degraded analogs are reacted with piperidine prior to analysis by GC-FID. The technique offers short analysis time, high resolving power, and high sensitivity. The derivatization reagent was selected based on nucleophilicity and its ability to react with both functionalities of CAC as well as CAA and MCA.

2. Experimental

2.1. Materials

Chloroacetyl chloride (≥99%), chloroacetic acid (≥99%), methylchloroacetate (99%), piperidine (99%), 1,2-dichoroethane (≥99%), and acetonitrile (HPLC Ultra grade) were obtained from Sigma-Aldrich (St. Louis, MO, USA). De-ionized water was from an in-house Milli-Q water purification system (Millipore, Billerica, MA). Individual stock solutions were prepared in dichloroethane at a concentration of 200 μg/mL. Working standard solutions were prepared at different concentration by diluting the respective stock solution with dichloroethane.

Table 1
Chromatographic conditions.

Parameter	Setting		
Column	Agilent HP-1, 30 m x 0.32 mm, 0.25 μm		
Inlet Temperature (°C)	260		
Split Ratio	25:1		
Injection Volume (μL)	1		
Column Flow (mL/min)	Helium at 1.0 mL/min (constant flow)		
Oven Temperature (°C)	140		
Oven Temperature	Ramp	Hold Time	Final Temp
Program (°C/min)	N/A	0	140
	20	2	300
Total Run time (min)	10		
Detector Temperature (°C)	300		
Detector Gas Flow	Hydrogen		30
(mL/min)	Air		400
	Makeup (He)		30

2.2. Derivatization

For the standard preparation, 2 mL of the working standard solution in dichloroethane was transferred into a scintillation vial and placed in an ice bath. Neat piperidine (0.5 mmol) was added dropwise to the solution at 0 °C. The solution was stirred on an orbital shaker and heated at 35 °C using a heating block. After 3 h, the solution was removed, filtered on 0.2 μm PTFE filter and analyzed by GC-FID. Chemical structures for the different compounds as well as their respective derivatives are shown in Fig. 1. For CAC, the objective was to drive the derivatization reaction to completion in order to only have the doubly substituted product and avoid complicated calculations from the two forms. In this study, MCA, CAA and CAC were determined at two different steps of the synthesis, including in the intermediate and in the final API product. For these pharmaceutical samples, a 2 mg/mL solution sample (intermediate or API) was prepared in dichloroethane, 2 mL was transferred into a scintillation vial and the subsequent steps were repeated.

2.3. Gas chromatography flame ionization detection (GC-FID) and confirmation by mass spectrometry (GC–MS)

The GC-FID system was an Agilent 7890A series gas chromatograph equipped with split/splitless injector and a flame ionization detector. The optimized conditions can be found in Table 1. Confirmation of the reaction products was performed using GC–MS under same conditions as previously described. The GC–MS system was an Agilent 7890A series gas chromatograph equipped with split/splitless injector and a mass spectrometry detector MSD 5975C, including an electron impact ionization source. The molec-