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Trace level detection and quantitation of ethyl diazoacetate by reversed-phase high performance liquid chromatography and UV detection

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Abstract

A method using reversed-phase high performance liquid chromatography (HPLC) with UV detection has been developed and validated for the trace level (ng/mL) detection and quantitation of ethyl diazoacetate (EDA), a toxic impurity, in sample matrix. Method development included the evaluation of several analytical techniques including LC–MS and GC–MS, which in this case, proved to be unacceptable means of analysis. The chromatographic separation employed in this method utilizes a mobile phase system of acetonitrile and water with analysis carried out using UV detection at 250 nm. The final method showed excellent linearity, accuracy, repeatability, specificity and recovery when evaluated at the quantitation limit (QL) of 6 ng/mL.

Keywords: Ethyl diazoacetate; Trace level detection; Cyclopropanation; HPLC; UV detection; Diazo compounds

1. Introduction

Ethyl diazoacetate (EDA) (**I**) (Fig. 1) is often used as a carbenoid or carbene precursor for the cyclopropanation of alkenes [1]. Reactions involving its use can be found in the pharmaceutical industry for the formation of compounds used as synthetic starting materials, intermediates or final active pharmaceutical ingredient (API) [2]. Ethyl diazoacetate is known to be toxic (oral: rat, median lethal dose 400 mg/kg; intravenous: rat, median lethal dose 280 mg/kg) and is thought to be a potential carcinogen/mutagen [3,4]. If this compound were used in or associated with a synthetic route and there was concern that it may not be eliminated during the processing steps of the reaction, its residual content should be investigated at a toxicologically acceptable level. Such trace level determinations may be difficult.

Although the use of EDA in synthetic organic chemistry is well documented [1], trace level detection is not; probably in part due to its reactivity. EDA is part of a class of compounds, which are known to be very reactive. It is known to be heat sensitive, emit toxic fumes and has the potential to explode when heated [3]. Work on EDA, including detonation properties and thermal stability, has been performed to show that it is safe for large scale use in pilot plant facilities [5–8]. Results based on thermal stability data indicate that EDA does not show a proclivity for detonation [5]. Work on EDA decomposition has been carried out using head space gas chromatography (HS-GC) and results indicate that its major decomposition products include carbon dioxide, ethane and nitrogen [6]. Other work has shown that decomposition results primarily in the formation of high boiling esters [6,7]. The onset temperature at which this decomposition occurs can be as low as 104 °C at a heating rate of 1 °C/min as measured by differential scanning calorimetry (DSC) [9]. However, accelerated rate calorimetry (ARC) data suggests an onset decomposition temperature as low as 55 °C (97 wt%) and is proportional to the EDA concentration [6].

This paper describes the development and validation of a reversed-phase liquid chromatographic (LC) method with UV detection for trace level detection and quantitation of EDA, a toxic and potentially carcinogenic/mutagenic carbenoid or carbene precursor for the cyclopropanation of alkenes. The initial approaches investigated for method development are also

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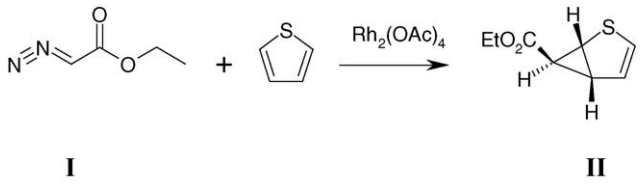

Fig. 1. Cyclopropanation scheme containing the structures of ethyl diazoacetate (**I**) and cyclopropanated alkene (**II**) sample matrix.

described and compared which include gas chromatography-mass spectrometry (GC-MS), GC using cool-on column injection (OCI) and reversed-phase high performance liquid chromatography-mass spectrometry (HPLC-MS) using electrospray ionization (ESI) in both the positive and negative modes.

2. Experimental

2.1. Chemicals

Ethyl diazoacetate (containing ≤10% dichloromethane) and ammonium acetate were purchased from Sigma–Aldrich Chemical Company Incorporated (St. Louis, MO, USA). HPLC grade acetonitrile, water and methanol were purchased from Honeywell Burdick and Jackson (Muskegon, MI, USA). GC grade ethyl acetate was purchased from Mallinckrodt Baker Inc. (Phillipsburg, NJ, USA). A sample of compound **II** (Fig. 1) was obtained from the Chemical Product Research and Development Laboratories at Eli Lilly & Company (Indianapolis, IN, USA).

2.2. Standard stock solution preparation

For HPLC analysis and method validation, a 1 mg/mL stock solution of EDA was prepared in a mixture of water and acetonitrile (85:15, v/v). Linearity standards were prepared by dilution from this single stock solution. Samples for HPLC-MS evaluation were prepared at a concentration of 1.0 mg/mL in acetonitrile and at 10 mg/mL in a mixture of 3 mM NH_4OAc in methanol/water (80:20, v/v). The GC-MS samples were prepared at a concentration of 11 mg/mL in a mixture of water and acetonitrile (60:40, v/v). For OCI, a 2.6 mg/mL stock solution of EDA was prepared in ethyl acetate. Linearity standards were prepared by dilution from this single stock solution.

2.3. Spike and recovery stock solutions and sample preparation

Three concentrations of EDA were prepared as stock solutions (62.0; 247.8; 1239.0 ng/mL). These solutions were then spiked 1:10 into each one of 18 solutions containing the sample matrix (Compound **II**, Fig. 1). A total of six solutions per concentration level were prepared. The sample matrix solutions were prepared at a concentration of 10 mg/mL using solid compound of 99.4% purity (Compound **II**, Fig. 1). Dissolution of this solid material was accomplished with a mixture of acetonitrile and water (60:40, v/v).

Table 1
HPLC conditions for the analysis of EDA

Column	C_{18} 4.6 × 150 mm 3.5 μm
Flow rate (mL/min)	1.5
Wavelength (nm)	250
Column temperature (°C)	25
Injection volume (μL)	10.0
Gradient[a]	
0 min	85% A and 15%B
10 min	30% A and 70% B
11 min	85% A and 15% B
15 min	85% A and 15% B
Total run time (min)	15.0

[a] Mobile phase A: water; mobile phase B: acetonitrile.

2.4. Instrumentation and software

2.4.1. HPLC and HPLC-MS

All liquid chromatographic analysis was conducted on an Agilent 1100 series HPLC equipped with a UV–vis variable wavelength detector set at 250 nm, Agilent Technologies Inc. (Palo Alto, CA, USA). Separations were obtained on a SunFire™ C_{18} column (4.6 mm × 150 mm 3.5 μm), Waters Corporation (Milford, MA, USA). The remaining HPLC conditions are detailed in Table 1. The data were collected via TOTALCHROM™ Version 6.2, PerkinElmer™ Instruments LLC (Shelton, CT, USA). The HPLC-MS analysis was conducted on an Agilent 1100 series HPLC, equipped with a photo diode array detector (PDA), Agilent Technologies Inc. (Palo Alto, CA, USA). The instrument was coupled to a Micromass ZMD, single quadrupole mass spectrometer, operating in positive and negative electrospray ionization mode, Micromass UK Ltd. (Manchester, UK). Separations were obtained on an Xterra® MS C_{18} (2.1 mm × 50 mm 3.5 μm) column, Waters Corporation (Milford, MA, USA). These data were collected with MassLynx Software, Version 3.5, Micromass UK Ltd. (Manchester, UK).

2.4.2. OCI and GC-MS

Analysis utilizing OCI was performed on an Agilent 6890N series GC equipped with a flame ionization detector (FID), Agilent Technologies Inc. (Palo Alto, CA, USA). Separations were obtained on an Agilent DB-1701 (30 m × 0.25 mm i.d. × 0.25 μm film) GC column preceded by an Agilent FS, deactivated (0.53 mm i.d. × 1 m) retention gap, Agilent Technologies Inc. (Palo Alto, CA, USA). The remaining OCI conditions are detailed in Table 2. The data were collected via TOTALCHROM™ Version 6.2, PerkinElmer™ Instruments LLC (Shelton, CT, USA). The GC-MS analysis was conducted on an Agilent series 6890 GC linked to a 5973N MSD mass spectrometer, Agilent Technologies Inc. (Palo Alto, CA, USA). Separations were obtained on an Agilent DB-Wax (30 m × 0.25 mm i.d. × 0.25 μm film) GC column, Agilent Technologies Inc. (Palo Alto, CA, USA). These data were collected via MSD Chemstation Software, also by Agilent Technologies Inc.

2.4.3. UV–vis spectroscopy

UV measurements were determined using an Hewlett-Packard (HP) 8453A UV–vis spectrophotometer, Agilent