Chemistry Specifications for Chemistry Services Contractors

National Toxicology Program

Appendix 3.1

Vehicle Analysis of Ethanol

September 20, 2013

Dose Vehicle Analysis of Ethanol

NIEHS Contract No. N01-ES-55385

ETP Task No. CHEM02363

MRI Project No. 4300

MRI Task No. 792

Midwest Research Institute

425 Volker Boulevard

Kansas City, Missouri 64110

January 29, 1997

Ethanol

CAS No.: 64-17-15	Lot No.: 96J31K
Lab Chemical ID Code: N/A	MRI Assigned Batch No.: 01
Internal Task No.: 792	Amount Received: 8 × 1-gallon plastic jugs
ETP Task No.: CHEM02363	Sample Receipt Date: 12/05/1996
Program Supported: GPS	Appearance: Clear, colorless liquid
Analysis Dates: 12/13-20/1996	Storage Conditions: Ambient
Initiation Date: 12/12/1996	Vendor: McCormick Distilling Co., Inc.
Interim Result Date: N/A	Vendor Purity: 95%

Structure	Molecular Weight	Molecular Formula
H_3CCH_2OH	46.07	C_2H_6O

Quality Assurance Statement

Dose Vehicle Analysis of Ethanol

ETP Task No. CHEM02363
MRI Project No. 4300
MRI Task No. 792

This study was inspected by the Quality Assurance Unit of MRI (QAU) and the findings reported to the Study Director and Management as follows:

Phase Inspected	Date Inspected	Date Reported
Protocol Audit	12/17/1996	12/18/1996
In-life Audit, GC Purity Assessment	12/17/1996	12/18/1996
Data Audit	1/24/1997	1/24/1997
Report Audit	1/24/1997	1/24/1997

This report reflects the procedures and raw data generated in this study. The raw data and report will be stored in the MRI Archives.

In addition to the study-specific audits/inspections cited above, inspections of the applicable facilities and equipment were performed by the QAU and reports were submitted to management as follows:

Facility/Equipment	Inspection Date	Management Submittal Date
PCA Laboratory Complex	7/31/1996	8/2/1996
Gas Chromatography Facility	8/20/1996	8/26/1996

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[redacted]

Carlos Castro
Senior Quality Assurance Officer

Approved:

[redacted]

Eugene G. Podrebarac, Ph.D.
Manager, Quality Assurance

January 29, 1997

Good Laboratory Practice Compliance Statement

This study was conducted in compliance with the Good Laboratory Practice regulations of the U.S. Food and Drug Administration (21 *CFR* 58).

Michael Kozak
Study Director

[redacted]

Date 1/30/97

Executive Summary

The purpose of this task was to verify the identity and assess the purity for a sample of ethanol. The identity of ethanol was confirmed by infrared and ^{1}H NMR spectroscopy. No impurities were detected by NMR spectroscopy, and none were observed by gas chromatography at $ 0.05% relative to the major component.

Contents

Figures

Dose Vehicle Analysis of Ethanol

1 Introduction

The purpose of this task was to verify the identity and assess the purity of a sample of ethanol. The identity of the test article was verified by infrared spectroscopy. Nuclear magnetic resonance spectroscopy (NMR) was used to detect impurities as well as to confirm identity. Impurities greater than or equal to 0.05% were assessed by gas chromatography, by comparison of an approximately 0.1% ethanol solution in water with neat 95% ethanol.

2 Chemical Information

Test Article:	Ethanol
Lot No.:	96J31K
MRI Assigned Batch No.:	01
Supplier:	McCormick Distilling Co. Inc.
Molecular Formula:	C_2H_6O
CAS No.:	64-17-5

3 Analysis Methods

3.1 Identity by Infrared Spectroscopy

3.1.1 Sample Preparation

The sample was prepared by placing a fraction of a drop of ethanol on a silver chloride plate which was then covered by a second plate. The plates were placed in a cell holder and the sample was scanned.

3.1.2 Instrument

Analect RFX-75 FT-IR, with the following parameters.
Scan Range: 400 cm^{-1} to 4400 cm^{-1}
Number of Scans: Background: 256; Sample: 256
Nominal Resolution: Data: 8 WN; Display: 8 WN
Apodization: Norton-Beer (medium)
Phase Correct: 1024-point Mertz

3.1.3 Analysis

The spectrum of the sample was obtained from 400 to 4400 cm^{-1}.

3.1.4 Results

The FT-IR spectrum of the sample is contained in Figure 1, attached. The sample spectrum was consistent with the literature spectrum[1] for ethanol.

3.2 Nuclear Magnetic Resonance Spectroscopy

3.2.1 Sample Preparation

The sample was prepared for analysis by adding 21.69 mg of ethanol, 1 mL of deuterated chloroform and a fraction of a drop of tetramethylsilane into a vial. The solution was mixed and a portion was pipetted into an NMR tube for analysis. After a spectrum was obtained, a drop of deuterium oxide was added to the NMR tube and the tube was inverted and allowed to settle. A second spectrum was then obtained.

3.2.2 Instrument

Varian VXR-300 FT-NMR with VXR-4000 data system

3.2.3 Analysis

A proton spectra was obtained over the range of 0 to 15 ppm. A second spectrum was obtained after the D_2O exchange.

3.2.4 Results

The FT-NMR spectrum of the sample is contained in Figure 2, attached. The sample spectrum was consistent with the literature spectrum[2] for ethanol.

[1] *The Aldrich Library of FT-IR Spectra*, Edition 1, Volume 1, Aldrich Chemical Company, United States of America, 1985, page 109, spectrum C

[2] *The Aldrich Library of C and H FT-NMR Spectra*, Edition 1, Aldrich Chemical Company, United States of America, 1993, page 163, spectrum B.

CH_3CH_2OH

(a) (c) (b)

Assignments (δ ppm)	Multiplicity	Observed Integration	Theoretical Integration
(a) 1.23	t	2.85	3
	$J_{(a,c)} = -7$ Hz		
(b) 2.09	s	1.56	1
(c) 3.71	q	2.15	2

3.3 Purity Assessment by Gas Chromatography

3.3.1 Sample Preparation

Two solutions were prepared and analyzed. A stock solution was prepared by volumetrically pipetting 1 mL of ethanol and 1 mL of cyclohexanone into a 100-mL volumetric flask. The contents of the flask were brought to volume using ASTM Type I reagent grade water and mixed by inversion. Solution A was prepared by delivering 1 mL of the stock to a 10-mL volumetric flask, diluting to volume with water, and mixing by inversion. Solution B was prepared by filling a 50-mL volumetric flask to the mark with ethanol. A 50-μL portion of cyclohexanone was added to the flask by Eppendorf pipet. The contents of the flask were mixed thoroughly.

3.3.2 Internal Standard Blank

An internal standard blank was prepared by volumetrically pipetting 1 mL of cyclohexanone into a 100-mL volumetric flask. The contents of the flask were brought to volume using water and mixed by inversion. This solution was volumetrically diluted 1 mL to 10 mL with water and mixed thoroughly.

3.3.3 Instrument

Varian 3700 with Varian 8000

Autosampler Injection Volume: 1 μL

Mode: Direct

Electronic Integration: TurboChrom for Windows version 4.0

Column: Supelcowax 10, 30 m • 0.53 mm ID, 1-μm film thickness, fused silica

Temperature Program: 100EC (5-min hold) to 220EC (3-min hold) at 10EC/min

Detector: Flame ionization

Attenuation: 32×10^{-11}

Temperatures:

Inlet: 110ºC

Detector: 220ºC

Carrier Gas: Helium

Flow Rate: 10 mL/min

Makeup Gas: Nitrogen

Flow Rate: 30 mL/min

Air Flow Rate: 300 mL/min

Hydrogen Flow Rate: 30 mL/min

3.3.4 Analysis

A portion of solution A, solution B, the internal standard blank, a water blank, and a sample of neat ethanol were placed in individual autosampler vials for analysis. Solutions A and B, the neat ethanol, and each of the blanks were injected at least in triplicate.

3.3.5 Results

3.3.5.1 System Suitability

The analytical system described in Section 3.3.3 was evaluated for reproducibility, theoretical plates, and tailing factor, according to USP guidelines[3]. Reproducibility was calculated using the average ethanol area obtained from 6 injections of solution A. Theoretical plates and tailing factor were evaluated at 5% base height for the ethanol peak of a single injection of solution A.

Reproducibility	Theoretical Plates	Tailing Factor
3378823 ± 97071 (s) %RSD = 2.9	4350	2.20

3.3.5.2 Chromatographic Profile

Impurity profiles were obtained for the test article using the system described in Section 3.3.3. Due to difficulties encountered while integrating the internal standard peak areas, the internal standard peak was not used in calculations. The average ethanol area was determined for 3 injections of solution A. The average area of each impurity peak not observed in the blank injections was calculated for the neat ethanol injections. The average relative percent was calculated for each impurity by comparing the average impurity area from the solution A injections corrected for the dilution factor (1000). No impurities were observed with areas ≥ 0.05% relative to the corrected ethanol area. The chromatograms are illustrated in Figure 3, attached.

[3] *The United States Pharmacopeia*, Twenty-second Revision, (USP XXIII), Physical Tests/ Chromatography 621, System Suitability, (1995) pp 1776

4 Summary

The test article was identified as ethanol by spectroscopy. No impurities were observed with relative area ≥ 0.05%.

5 Contributors

Mr. Duane Stephens and Mr. Jason McClintock contributed to this study.

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[redacted]

Michael Kozak [redacted]
Study Director

Approved:

[redacted]

Robert E. Smith, Ph.D.
Principal Investigator

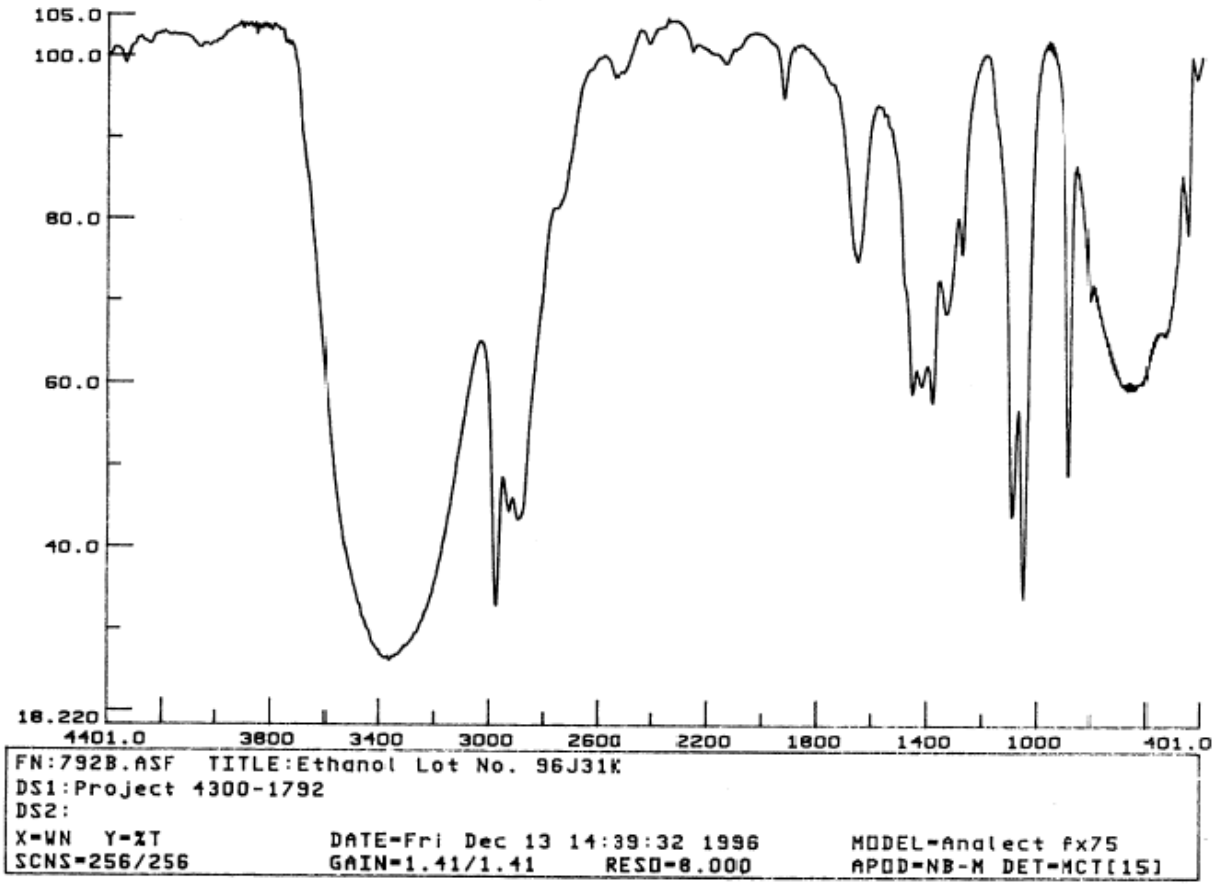
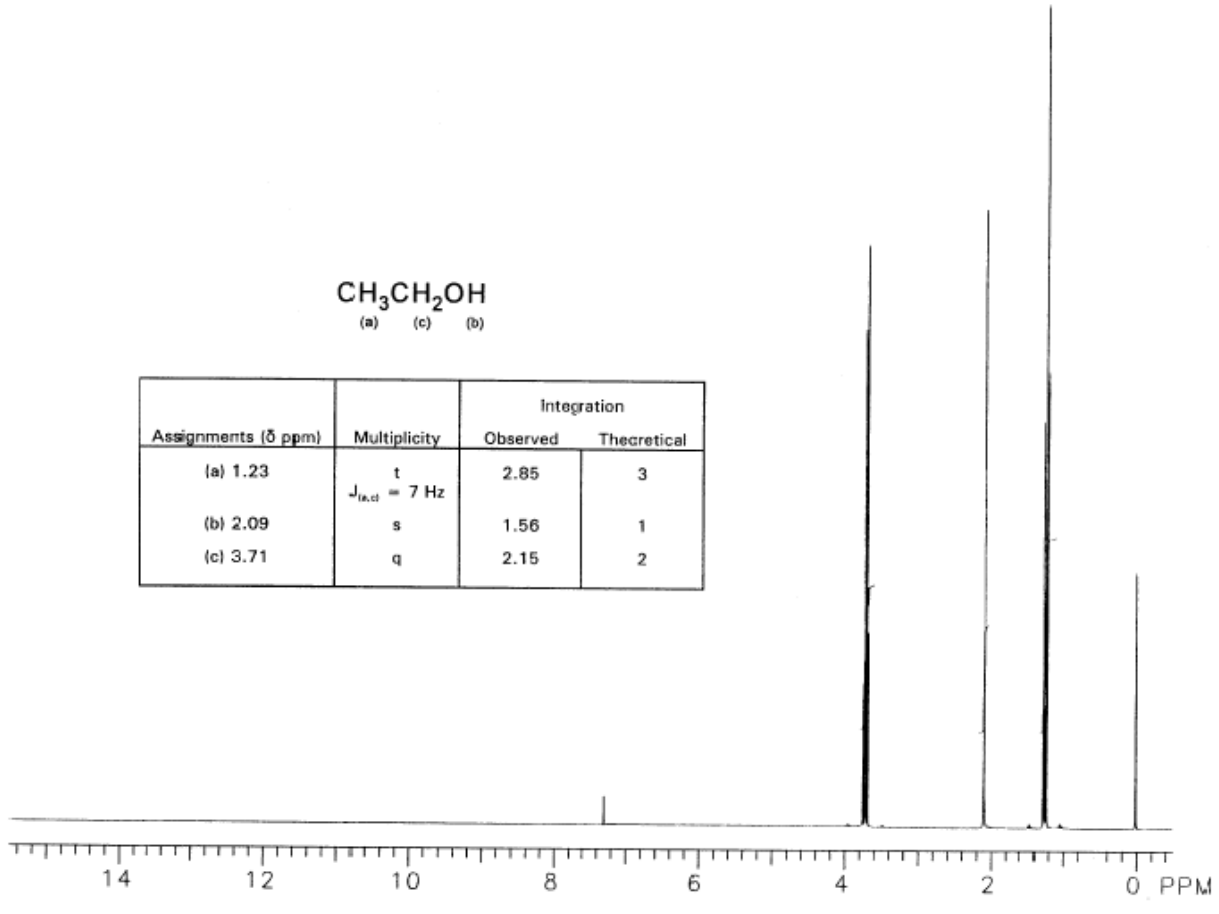


Figure 1. Fourier Transform Infrared Spectrum of Ethanol (Lot No. 96J31K)



Assignments (δ ppm)	Multiplicity	Integration	
		Observed	Thecretical
(a) 1.23	t $J_{(a,c)}$ = 7 Hz	2.85	3
(b) 2.09	s	1.56	1
(c) 3.71	q	2.15	2

Figure 2. Fourier Transform Nuclear Magnetic Resonance Spectrum of Ethanol (Lot No. 96J31K)

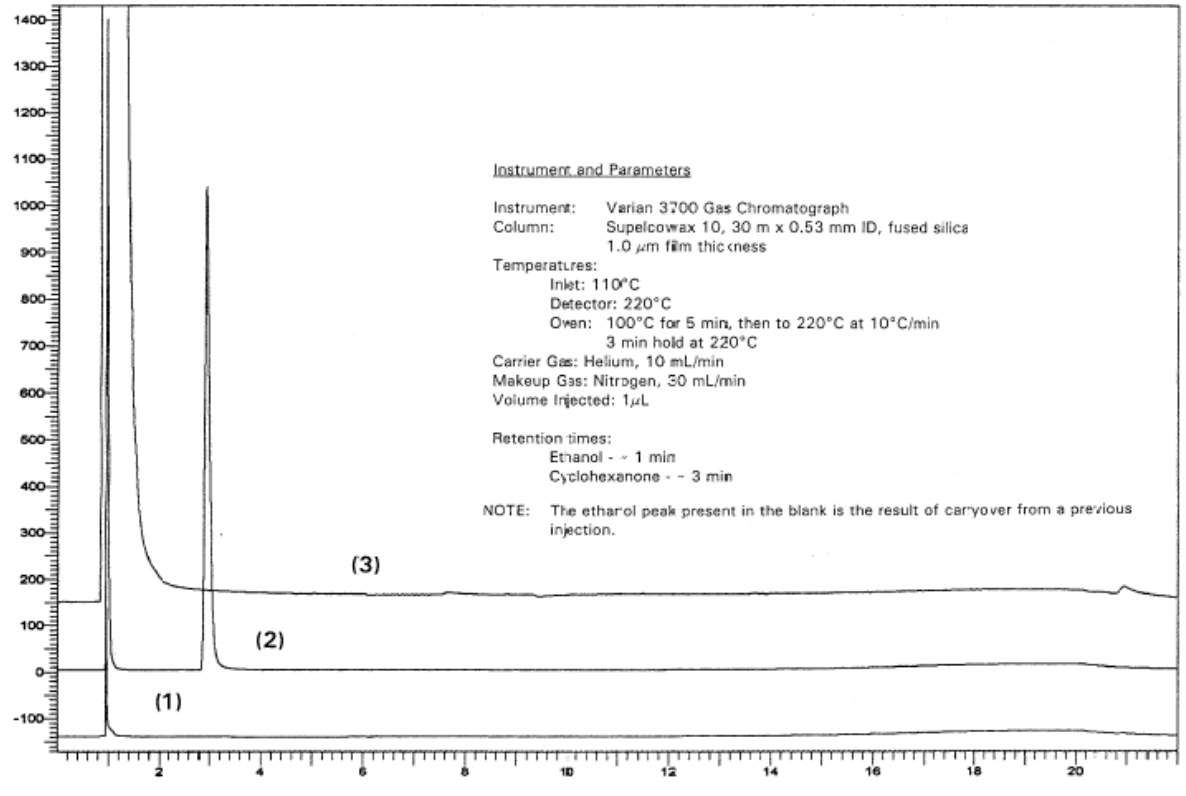


Figure 3. Typical Chromatograms for Analysis
(1) Blank. (2) Solution A (3) Ethanol (Lot No. 96J31K)