

Determination of Succinic, Glutaric, and Adipic Acids as Quality Control of Cyclohexanone Production

INTRODUCTION

Cyclohexanone is the key intermediate for the production of adipic acid, an important compound for the synthesis of nylon 66.¹ Cyclohexane oxidation is the normal method for the production of cyclohexanone; and succinic, glutaric, and adipic acids (structures shown in Figure 1), as well as their dehydration products, are the main by-products.² The three aliphatic dicarboxylic acids are important chemical raw materials as well, and their determination is important for the quality control of cyclohexanone production and recycling of the by-products.

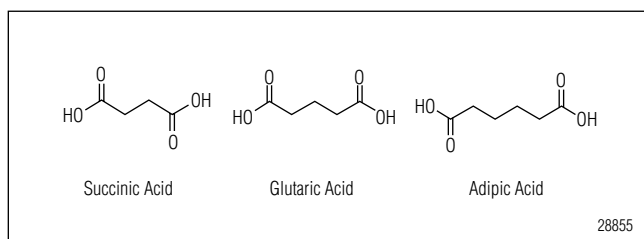


Figure 1. Structures of succinic, glutaric, and adipic acids.

It is difficult to use potentiometric titration to determine succinic, glutaric, and adipic acids due to their similar ionization constants.³ To determine these compounds by gas chromatography (GC), precolumn derivatization—esterification with alcohols—is required because of their strong polarity and high boiling point.⁴ This is a time-consuming and complicated operation, resulting in poor reproducibility of the esterification rate. Therefore, the determination of succinic, glutaric, and adipic acids is usually performed by ion chromatography (IC)⁵⁻⁷ and reversed-phase high-performance liquid chromatography (RP-HPLC).^{8,9} Because the three aliphatic dicarboxylic acids lack a good ultraviolet (UV) chromophore, mass spectrometry (MS) is used to achieve sensitive detection.^{10,11}

The work shown here describes an LC-MS method for the determination of succinic, glutaric, and adipic acids in the by-products of cyclohexanone production. The separation is performed on a Thermo Scientific Acclaim™ Organic Acid (OA) column, which is designed for the separation of organic acids.^{12,13} Baseline separation of these three aliphatic dicarboxylic acids is observed with resolutions (R_s) ≥ 2.6 and completed within 10 min. Analytes are detected by electrospray ionization (ESI) on a Thermo Scientific MSQ Plus™ mass detector operated in the negative ion mode by full scan MS. The mass spectra of the peaks eluting at approximately 1.8, 2.5, and 4.5 min reveals the deprotonated molecular ions [M-H]⁻ at m/z 116.9, m/z 130.9, and m/z 144.9 for succinic, glutaric, and adipic acids, respectively.

Ions with masses of m/z 72.9, m/z 86.9, and m/z 100.9 are characteristic fragment ions caused by in-source collision induced dissociation (CID) and correspond to the loss of one carboxyl group using a cone voltage setting of 60 V.

Quantification is accomplished via selected ion monitoring (SIM) using ions corresponding to each of the dicarboxylic acids at m/z 117 for succinic acid, m/z 131 for glutaric acid, and m/z 145 for adipic acid. Excellent linearity within 0.1 to 5 mg/L is observed for each dicarboxylic acid, and method detection limits (MDL) are all ≤ 0.06 mg/L.

EQUIPMENT

Thermo Scientific Dionex UltiMate™ 3000 RSLC system including:

SR-3000 without Degasser
LPG-3400RS Pump
WPS-3000TRS Autosampler
TCC-3000RS Thermostatted Column Compartment
DAD-3000RS UV-vis Detector

MSQ Plus mass detector with ESI source

Thermo Scientific Dionex AXP-MS Pump

Thermo Scientific Dionex Chromeleon™ Chromatography Data System (CDS) software, Version 6.80, SR9 or higher

REAGENTS AND STANDARDS

Deionized (DI) water, from Milli-Q® Gradient A10

Acetonitrile (CH₃CN), HPLC grade
(Cat.#AC610010040), Fisher Chemical

Formic acid (FA), HPLC grade, SCRC, Shanghai, China

Succinic, glutaric, and adipic acids (analytical grade) were purchased from SCRC, Shanghai, China.

Prepare stock standard solutions with concentration of 1000 mg/L for each aliphatic dicarboxylic acid by dissolving the appropriate amount of standards in DI water. Prepare four working standard solutions ranging from 0.1 to 5.0 mg/L for the calibration by adding the appropriate amount of the stock standard solution and diluting with DI water.

SAMPLE PREPARATION

Three sample solutions of mixed dibasic acids (#1, #2, and #3) generated from cyclohexane production were kindly donated by a customer located in Jiangsu Province, China.

Make thousand-fold dilutions with DI water and filter through a 0.45 μ m membrane (Millex®-LH) prior to injection.

CHROMATOGRAPHIC CONDITIONS

Analytical Column: Acclaim OA, 3 μ m,
2.1 \times 150 mm (P/N 070087)

Mobile Phase: A: 0.1% FA aqueous (v/v)
B: CH₃CN
In gradient
-5.0–0.0 min, 5% B,
0.0–5.0 min, 5–40% B

Flow Rate: 0.3 mL/min

Inj. Volume: 10 μ L

Column Temp.: 30 °C

MSQ-Plus Mass Detector Conditions

Ionization Interface: ESI

Operating Mode: Negative Scan

Scan Events: Full scan for identification:
65–150 m/z
SIM scan for quantitation: succinic
acid: 117 m/z ; glutaric acid: 131 m/z ;
adipic acid: 145 m/z

Probe Temp.: 400 °C

Needle Voltage: 3.5 KV

Cone Voltage: 60 V

Nebulizer Gas: Nitrogen at 75 psi

RESULTS AND DISCUSSION

Separation of Succinic, Glutaric, and Adipic Acids on the Acclaim OA Column

Formic acid was used in place of sulfate or phosphate in order to develop an MS-compatible method with comparable performance to typical ionic mobile phases. Figure 2 shows the chromatograms of a mixed standards solution and an undiluted sample solution separated on the Acclaim OA column under the specified chromatographic conditions with UV detection. Succinic, glutaric, and adipic acids were separated with good resolution, and two unknown peaks with similar UV spectra to the three dicarboxylic acids (Figure 3) were found in the sample solution. The purity of these peaks may be estimated using the peak purity match factor, which can be calculated by Chromeleon CDS software. The calculated values are 999 for succinic, 1000 for glutaric, 998 for adipic acids, 994 for unknown peak 1, and 997 for unknown peak 2 (the corresponding value for 100% purity is 1000). These results demonstrate that the Acclaim OA column provides good selectivity and is suitable for the determination of succinic, glutaric, and adipic acids in these sample solutions.

Further experimentation showed that the two unknown compounds with UV absorption showed no MS response. Efforts to ionize and detect the unknowns by MS (i.e., enlargement of scan range from 50 to 800 amu, application of different cone voltages between 20 and 100 V, use of atmospheric-pressure chemical ionization [APCI] and ESI sources in negative and positive ion modes, and use of mobile phases with different pH values) were unsuccessful. Because the unknowns were generated during cyclohexane oxidation, they may be dehydration products of the three dicarboxylic acids² and/or other degradants of cyclohexanone.

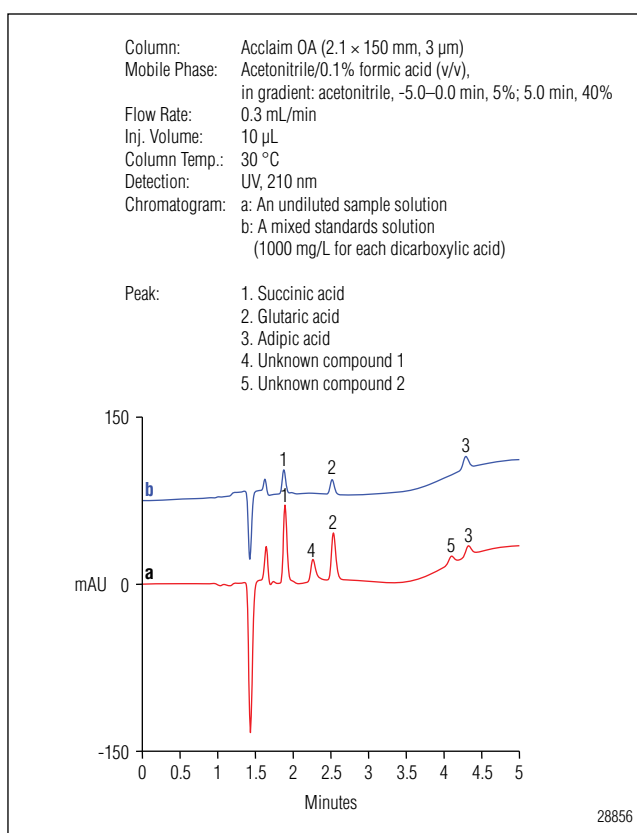


Figure 2. Chromatograms of (a) an undiluted sample solution and (b) a mixed standards solution (1000 mg/L for each dicarboxylic acid).

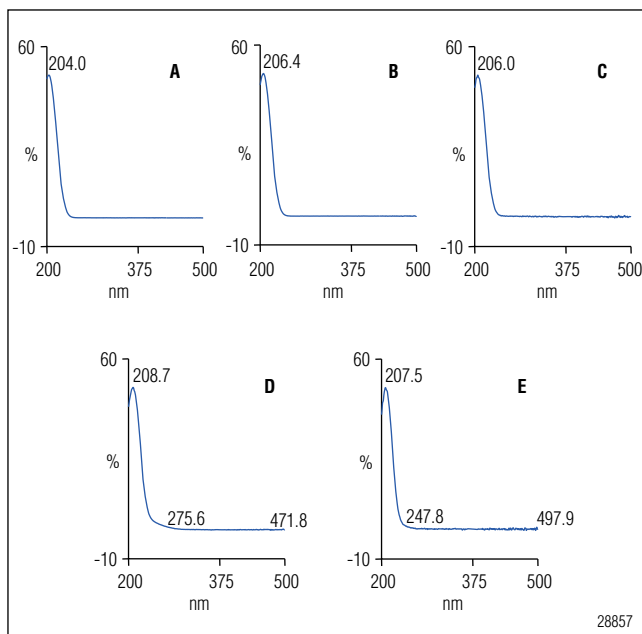


Figure 3. UV spectra of (A) succinic acid, (B) glutaric acid, (C) adipic acid, (D) unknown compound 1, and (E) unknown compound 2.

Method Reproducibility, Linearity, and Detection Limits

Method reproducibility was estimated by making seven consecutive 10 μL injections of a mixed dibasic acids sample spiked with 1.0 mg/L of each standard using MS detection. The retention time and peak area reproducibilities are summarized in Table 1 and show good precision.

Table 1. Reproducibility for Peak Retention Time and Area

Analyte	Retention Time RSD	Peak Area RSD	Standard Concentration (mg/L)
Succinic acid	0.000	2.412	1.0
Glutaric acid	0.270	1.313	1.0
Adipic acid	0.215	0.936	1.0

Calibration linearity for MS detection of succinic, glutaric, and adipic acids was investigated by making three consecutive injections of mixed standards prepared at four different concentrations. The external standard method was used to establish the calibration curve and to quantify these aliphatic dicarboxylic acids in the mixed dibasic acids samples. Excellent linearity was observed from 0.1 to 5 mg/L when plotting the concentration versus the peak area (obtained in SIM channels), and the coefficient of determination was ≥ 0.9980 for each plot.

The MDL of each aliphatic dicarboxylic acid for MS detection was calculated following the equation:

$$\text{Detection limit} = S \times t_{(n-1, 1-\alpha=0.99)}$$

The symbol S represents Standard Deviation (SD) of replicate analyses, n represents number of replicates, and $t_{(n-1, 1-\alpha=0.99)}$ represents Student's t value for the 99% confidence level with $n - 1$ degrees of freedom. Seven replicate injections of a mixed dibasic acids sample spiked with 1.0 mg/L of each standard with MS detection were used to determine the MDLs. Table 2 summarizes the calibration and MDL data, which show excellent method linearity and sensitivity.

Sample Analysis

Three mixed dibasic acid samples generated during cyclohexane oxidation for the production of cyclohexanone in different chemical factories were kindly supplied by a customer. Assays were carried out to determine the contents of succinic, glutaric, and adipic acid in the samples.

Table 2. Method Linearity Data and Method Detection Limits (MDL)*

Analyte	Regression Equations	r^2	MDL (mg/L)
Succinic acid	$A = 667.3 c + 21.86$	0.9993	0.04
Glutaric acid	$A = 1144 c + 139.0$	0.9980	0.06
Adipic acid	$A = 1787 c + 145.9$	0.9985	0.04

*The single-sided Student's t test method (at the 99% confidence limit) was used for determining MDL, where the standard deviation (SD) of the peak area of seven injections is multiplied by 3.71 to yield the MDL.

The customer planned to extract the three aliphatic dicarboxylic acids from the by-product solutions and use them as materials to make other chemical products. Figure 4 shows the Total Ion Current (TIC) chromatograms of the original and spiked samples.

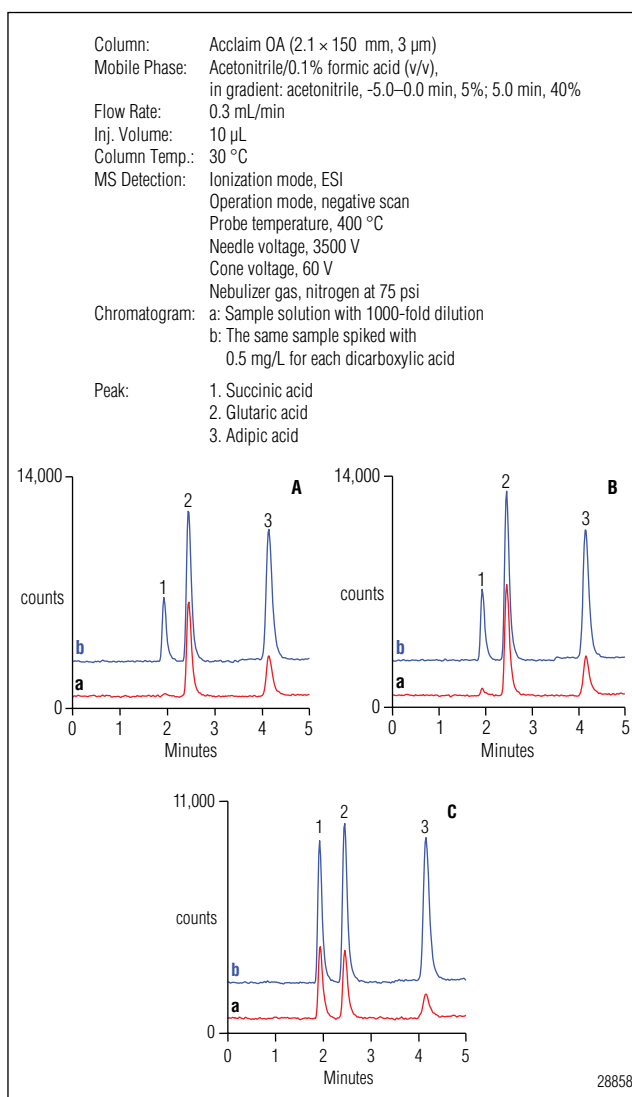


Figure 4. TIC chromatograms of sample solutions of mixed dibasic acids (A) #1, (B) #2, and (C) #3.

Figure 5 shows the mass spectrum of each individual acid in a mixed dibasic acids sample. The deprotonated molecular ions $[M-H]^-$ at m/z 116.9 for succinic acid, m/z 130.9 for glutaric acid, and m/z 145.0 for adipic acid were in good agreement with their theoretical molecular weights of 118.1 amu, 132.1 amu, and 146.1 amu, respectively. The ions detected at m/z 72.9, m/z 86.9, and m/z 100.9 are characteristic fragment ions caused by in-source collision induced dissociation (CID) and correspond to the loss of one carboxyl group $[M-44H]^-$ using a cone voltage setting of 60 V. Additional fragment ions were detected at m/z 98.9, m/z 112.9, and m/z 127.0 and correspond to the loss of one water molecule. The presence of molecular ions and characteristic fragment ions positively confirmed the identities of the three dicarboxylic acids in the sample. Table 3 reports the data for sample analysis as automatically generated by the Chromeleon software.

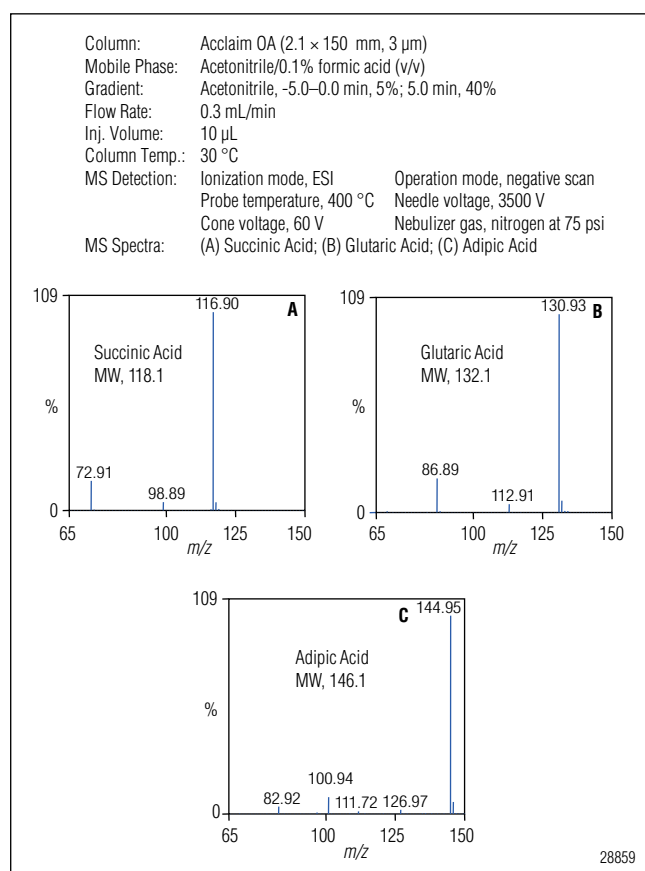


Figure 5. Mass spectra of (A) succinic, (B) glutaric, and (C) adipic acids from the chromatography of sample solution #2.

Table 3. Analysis Results for Succinic, Glutaric, and Adipic Acids in the Cyclohexanone Production Samples*					
Sample**	Analyte	Amount Detected in Sample (mg/L)	Added (mg/L)	Total Amount Found in the Spiked Sample (mg/L)	Recovery (%)
#1	Succinic acid	0.03***	0.50	0.66	126
	Glutaric acid	0.58		1.00	84
	Adipic acid	0.20		0.65	90
#2	Succinic acid	0.07***	0.50	0.68	122
	Glutaric acid	0.66		1.09	86
	Adipic acid	0.19		0.67	96
#3	Succinic acid	0.50	0.50	1.06	112
	Glutaric acid	0.33		0.81	96
	Adipic acid	0.11		0.58	94

*Average of three determinations

**1000-fold dilution

***Less than or close to the MDL (0.04 mg/L)

CONCLUSION

This work describes an effective method for the determination of succinic, glutaric, and adipic acids in solutions of mixed dibasic acids generated during cyclohexane oxidation. Using the Dionex UltiMate 3000 RSLC system with the Acclaim OA column and MS detection, a fast baseline separation of these three dibasic acids was achieved with good resolution values ($R_s \geq 2.6$), good method reproducibility, excellent linearity within 0.1 to 5 mg/L, and accurate results for industrial samples.

REFERENCES

1. Zhang, M.; Wei, J.; Bai, Y.J.; Gao, Y.; Wu, Y., Miao, Y.Q.; Shi, Z. Study of Clear Oxidation of Cyclohexanone to Adipic Acid Using Hydrogen Peroxide. *Chin. J. Org. Chem.* **2006**, *26*, 207.
2. Zhou, W.Y.; Hu, B.C.; Zhao, L.R.; Liu, Z.L. Qualitative and Quantitative Analysis of the By-Products in the Course of Cyclohexanone Production by HPLC-MS/MS. *J. Chin. Mass Spectr. Soc.* **2009**, *30*, 31.
3. *Product Manual for IonPac ICE-ASI*, Document No. 031181-07, p 17, Dionex Corporation, Sunnyvale, CA, 2006. www.dionex.com/en-us/webdocs/4341-031181-07_Manual_ICE-ASI_V30.pdf (accessed Jun 30, 2011).
4. Liu, J.; Chen, L.; Zhou, H. Determination of Dicarboxylic Acids in a New Compound Liquid Fertilizer by Gas Chromatography. *Chin. J. Chromatogr. (Se Pu)* **1999**, *17*, 95.
5. Tanaka, K.; Chikara, H.; Hu, W.Z., Hasebe, K. Separation of Carboxylic Acids on a Weakly Acidic Cation-Exchange Resin by Ion-Exclusion Chromatography. *J. Chromatogr., A* **1999**, *850*, 187.
6. Ng, K.L.; Paull, B.; Haddad, P.R.; Tanaka, K. Retention Modeling of Electrostatic and Adsorption Effects of Aliphatic and Aromatic Carboxylic Acids in Ion-Exclusion Chromatography. *J. Chromatogr., A* **1999**, *850*, 17.
7. *IonPac ICE-ASI Ion-Exclusion Column*, LPN 0814-03, Data Sheet for Dionex Corporation, Sunnyvale, CA, 2008. http://www.dionex.com/en-us/webdocs/4276-DS_IonPac_ICE-ASI_02Apr07_LPN0814_03.pdf (accessed Jun 30, 2011).
8. Destandau, E.; Vial, J.; Jardy, A.; Hennion, M.C.; Bonnet, D.; Lancelin, P. Robustness Study of a Reversed-Phase Liquid Chromatographic Method for the Analysis of Carboxylic Acids in Industrial Reaction Mixtures. *Anal. Chim. Acta* **2006**, *572*, 102.
9. Destandau, E.; Vial, J.; Jardy, A.; Hennion, M.C.; Bonnet, D.; Lancelin, P. Development and Validation of a Reversed-Phase Liquid Chromatography Method for the Quantitative Determination of Carboxylic Acids in Industrial Reaction Mixtures. *J. Chromatogr., A* **2005**, *1088*, 49.
10. Käkölä, J.; Alén, R. A Fast Method for Determining Low-Molecular-Mass Aliphatic Carboxylic Acids by High-Performance Liquid Chromatography–Atmospheric Pressure Chemical Ionization Mass Spectrometry. *J. Sep. Sci.* **2006**, *29*, 1996.
11. Käkölä, J.; Alén, R.; Pakkanen, H.; Matilainen, R.; Lahti, K. Quantitative Determination of the Main Aliphatic Carboxylic Acids in Wood Kraft Black Liquors by High-Performance Liquid Chromatography–Mass Spectrometry. *J. Chromatogr., A* **2007**, *1139*, 263.
12. *Product Manual for Acclaim Organic Acid (OA)*, Document No. 031996-02, Dionex Corporation, Sunnyvale, CA, 2009. www.dionex.com/en-us/webdocs/41786-Man-031996-02-Acclaim-OA-Jul09.pdf (accessed Jun 30, 2011).
13. *Acclaim Organic Acid (OA) HPLC Column*, LPN 1623-01, Data Sheet for Dionex Corporation, Sunnyvale, CA, 2004. www.dionex.com/en-us/webdocs/25925-Acclaim_OA_V22.pdf (accessed Jun 30, 2011).

Milli-Q and Millex are registered trademarks of Millipore Corporation.
All other trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries.

Speed · Simplicity · Solutions



Dionex Products

1228 Titan Way
P.O. Box 3603
Sunnyvale, CA
94088-3603
(408) 737-0700

North America

U.S./Canada (847) 295-7500

South America

Brazil (55) 11 3731 5140

Europe

Austria (43) 1 616 51 25 Benelux (31) 20 683 9768 (32) 3 353 4294
Denmark (45) 36 36 90 90 France (33) 1 39 30 01 10 Germany (49) 6126 991 0
Ireland (353) 1 644 0064 Italy (39) 02 51 62 1267 Sweden (46) 8 473 3380
Switzerland (41) 62 205 9966 United Kingdom (44) 1276 691722

Asia Pacific

Australia (61) 2 9420 5233 China (852) 2428 3282 India (91) 22 2764 2735
Japan (81) 6 6885 1213 Korea (82) 2 2653 2580 Singapore (65) 6289 1190
Taiwan (886) 2 8751 6655

www.thermoscientific.com/dionex



LPN 2893-01 PDF 8/11
©2011 Thermo Fisher Scientific, Inc.