(2)

AD

TECHNICAL REPORT 8903

# DETERMINATION OF DIMETHYL SULFOXIDE (DMSO), ETHANOL (ETOH), FORMAMIDE (F) AND GLYCEROL/FORMAL (GF) BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

ERNST E. BRUEGGEMANN
ALAN B. ROSENCRANCE





U S ARMY BIOMEDICAL RESEARCH & DEVELOPMENT LABORATORY
Fort Detrick
Frederick, MD 21701-5010

January 1989



U S ARMY MEDICAL RESEARCH & DEVELOPMENT COMMAND
Fort Detrick
Frederick, MD 21701-5012

SECURITY CLASSIFICATION OF THIS PAGE

# REPORT DOCUMENTATION PAGE

Form Approved
OMB No 0704-0188

| | |
|---|---|
| 1a REPORT SECURITY CLASSIFICATION<br>Unclassified | 1b RESTRICTIVE MARKINGS |
| 2a SECURITY CLASSIFICATION AUTHORITY | 3 DISTRIBUTION / AVAILABILITY OF REPORT<br>Approved for public release; distribution unlimited. |
| 2b DECLASSIFICATION / DOWNGRADING SCHEDULE | |
| 4 PERFORMING ORGANIZATION REPORT NUMBER(S)<br>Technical Report #8903 | 5 MONITORING ORGANIZATION REPORT NUMBER(S) |
| 6a NAME OF PERFORMING ORGANIZATION<br>U.S. Army Biomedical Research & Development Laboratory<br>6b OFFICE SYMBOL (If applicable)<br>SGRD-UBG-R | 7a NAME OF MONITORING ORGANIZATION |
| 6c ADDRESS (City, State, and ZIP Code)<br>Building 568, Fort Detrick<br>Frederick, MD 21702-5010 | 7b ADDRESS (City, State, and ZIP Code) |
| 8a NAME OF FUNDING / SPONSORING ORGANIZATION U.S. Army Biomedical Research & Development Lab<br>8b OFFICE SYMBOL (If applicable)<br>SGRD-UBG-R | 9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER |
| 8c ADDRESS (City, State, and ZIP Code)<br>Building 568, Fort Detrick<br>Frederick, MD 21702-5010 | 10 SOURCE OF FUNDING NUMBERS<br>PROGRAM ELEMENT NO / PROJECT NO / TASK NO / WORK UNIT ACCESSION NO |

11 TITLE (Include Security Classification)
(U) Determination of Dimethyl Sulfoxide (DMSO), Ethanol, (ETOH), Formamide (F), and Glycerol/ Formal (GF) by High Performance Liquid Chromatography (HPLC).

12 PERSONAL AUTHOR(S)
ERNST E. BRUEGGEMANN and ALAN B. ROSENCRANCE

| 13a TYPE OF REPORT | 13b TIME COVERED | 14 DATE OF REPORT (Year, Month, Day) | 15 PAGE COUNT |
|---|---|---|---|
| Technical | FROM 1/88 TO 5/88 | 1989 January 30 | 13 |

16 SUPPLEMENTARY NOTATION

| 17 COSATI CODES | | | 18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number) |
|---|---|---|---|
| FIELD | GROUP | SUB-GROUP | High Performance Liquid Chromatograph (HPLC), Dimethyl/ Sulfoxide (DMSO), Ethanol (ETOH), Formamide (F), Glycerol/ Formal (GF), Analytical Method |
| | | | |
| | | | |

19 ABSTRACT (Continue on reverse if necessary and identify by block number)

Liquid chromatographic (LC) methods have been developed for the determination of dimethyl sulfoxide (DMSO), ethanol (ETOH), formamide (F), and glycerol/formal (GF), in aqueous samples. The samples are directly injected onto a high performance liquid chromatograph (HPLC) without further sample preparation.

Separation of DMSO and F was achieved by using a C18 column and a mobile phase containing water/methanol. Separation of GS and ETOH was achieved by using a Dextro-Pak column and a mobile phase consisting of water. A programmable ultraviolet (UV) spectrophotometric detector was used to monitor the LC effluent for DMSO and F. A refractive index detector was used to monitor the LC effluent for ETOH and GF. The precision and accuracy of all methods are included in the report.

| 20 DISTRIBUTION / AVAILABILITY OF ABSTRACT | 21 ABSTRACT SECURITY CLASSIFICATION |
|---|---|
| ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS | Unclassified |
| 22a NAME OF RESPONSIBLE INDIVIDUAL<br>Ernst E. Brueggemann | 22b TELEPHONE (Include Area Code)<br>(301) 663-2340<br>22c OFFICE SYMBOL<br>SGRD-UBG-R |

DD Form 1473, JUN 86 Previous editions are obsolete. SECURITY CLASSIFICATION OF THIS PAGE

# NOTICE

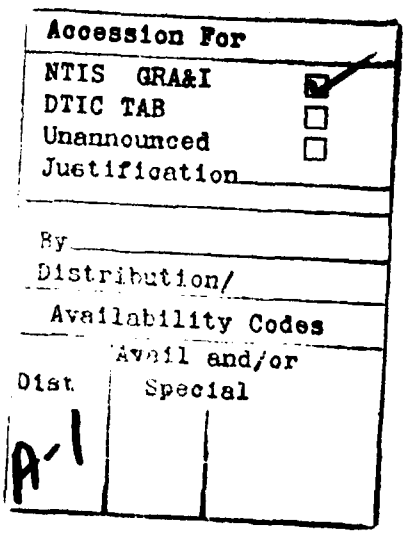
## Disclaimers

The views, opinions, and/or findings contained in this report are those of the author(s) and should not be construed as official Department of the Army position, policy, or decision, unless so designated by other official documentation.

Research was conducted in compliance with the Animal Welfare Act, and other Federal statues and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, NIH publication 86-23, 1985 edition.

## Disposition

Destroy this report when it is no longer needed. Do not return it to the originator.



## TABLE OF CONTENTS



### TABLES



### FIGURES

## INTRODUCTION AND OBJECTIVES

Currently, investigations are being performed at the U.S. Army Biomedical Research and Development Laboratory, Research Methods Branch, to determine the teratogenicity of ethanol, dimethyl sulfoxide, formamide and glycerol/formal on Xenopus laevis embryos.

In support of these investigations, it was our task to develop a separate High Performance Liquid Chromatographic (HPLC) method for each of these four compounds in aqueous solutions.

Although other analytical methods exist,[1-3] HPLC (employing reverse phase columns) appears to be the analytical method of choice because the water miscible samples can be directly injected onto the HPLC without any prior sample clean-up. In addition, different types of detectors can be easily interchanged in the HPLC system to detect the appropriate compound in solution.

Each HPLC method reported herein is intended to be quick, sensitive and reproducible for the determination of each compound in aqueous solution.

## METHODS AND MATERIALS

### ANALYTICAL INSTRUMENTATION

A Waters liquid chromatographic system, (Millipore Corp., Waters Chromatography Division, Milford, MA), was employed throughout the study. The system consisted of the following components: a model M6000A solvent delivery system, a model 710B WISP autosampler, a model 721 programmable system controller, a model 730 data module, a model R-400 refractive index detector, and a model 480 LC Spectrophotometer.

### CHEMICALS

The methanol used was "HPLC grade" from Burdick and Jackson Laboratories (Muskegon, MI). Reagent grade water was obtained with a Milli-Q System, (Millipore, Bedford, MA), and had a resistance of 18 megohms-cm.

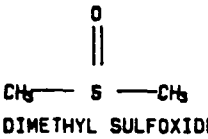
Dimethyl Sulfoxide (99 + % pure), glycerol/formal (98% pure) and formamide (99% pure) were obtained from the Aldrich Chemical Company, Inc., (Milwaukee, WI) and were used without further purification.

The ethanol (95 + % pure) used throughout this study was from U.S. Industrial Chemicals Co. (Tuscola, IL).

## CHEMICAL AND PHYSICAL PROPERTIES

### DMSO

CAS Registry Number: 67-68-5
RTECS Reference Number: PV6210000
M.W. 78.13
Chemical Formula: C2H6OS
Structure:

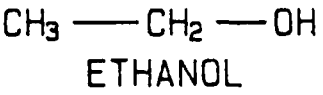


DIMETHYL SULFOXIDE

Synonyms: DEMESO; DEMASORB; DEMAVET; DERMASORB; DIMETHYL SULFOXIDE; DIMETHYL SULPHOXIDE; DMS-70; DMS-90; DOLIGUR; DOMOSO; DMSO; DROMISOL; GAMAL 90; HYADUK; INFILTRINA; METHANE, SULINYLBIS-; NSC-763; SOMIPRONT; SQ 9453; SYNTEXAN.[4]

### ETHANOL

CAS Registry Number: 64-17-5
RTECS Reference Number: KQ6300000
M.W.: 46.08
Chemical Formula: C2H6O
Structure:

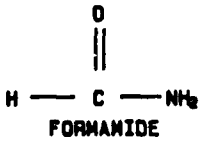


ETHANOL

Synonyms: Absolute Ethanol; Aethanol(GERMAN); Aethylalkohol (GERMAN); Alcohol; Alcohol Anhydrous; Alcohol dehydrated; Alcohol Ethylique(FRENCH); Alcool Etilico(ITALIAN); Algrain; Alkohol(GERMAN); Anhydrol; Cologne Spirit; Cologne spirits (alcohol)(DOT); Etanolo(Italian); Ethanol; Ethanol(DOT); Ethanol 200 proof; Ethyl Alcohol(DUTCH); Ethyl Alcohol Anhydrous; Ethyl Alcohol(DOT); Ethyl Hydrate; Ethyl Hydroxide, Etylowy Alkohol(POLISH); Fermentation Alcohol; Grain Alcohol; Jaysol S; Methylcarbinol; Molasses Alcohol; NA 1170(DOT); NCI-C03134; Potato Alcohol; Spirits of Wine; Spirit; Tecsol.[4]

### FORMAMIDE

CAS Registry Number: 75-12-7
RTECS Reference Number: LQ0525000
M.W.: 45.05
Chemical Formula: CH3NO
Structure:

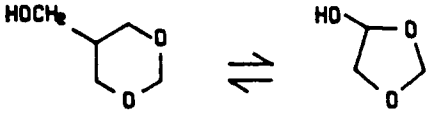


FORMAMIDE

Synonyms: Carbamaldehyde; Methanamide.[4]

## GLYCEROL/FORMAL

CAS Registry Number: 5464-28-8
RTECS Reference Number: JH8390000
M.W.: 104.12
Chemical Formula: C4H8O3
Structure:




GLYCEROL FORMAL

Synonyms: 1,3-dioxan-5-ol and 1,3-dioxolane-4-methanol(mixture); GF; glycerin-formale; sericosol-n.[4]

## HPLC CONDITIONS

### DMSO

Column: Microsorb C18 (Rainin, Inst. Co., Woburn.MA)
Mobile Phase: 3% methanol/water
Flow Rate: 2.0 mL/min.
UV: 220nm, .100 absorbance units full scale(aufs)
Injection Volume: 10 µL

### ETHANOL

Column: Dextro-Pak Column (Waters Chromatography Division, Milford, MA)
Mobile Phase: reagent grade water
Flow Rate: 1.0 mL/min.
Refractive Index: 8X (attenuation)
Injection Volume: 20 µL

### FORMAMIDE

Column: Microsorb C18 (Rainin Inst., Co., Woburn, MA)
Mobile Phase: reagent grade water
Flow Rate: 2.0 mL/min.
UV: 220nm, .100 absorbance units full scale(aufs)
Injection Volume: 10 µL

### GLYCEROL/FORMAL

Column: Dextro-Pak Column (Waters Chromatography Division, Milford, MA)
Mobile Phase: reagent grade water
flow rate: 1.0 mL/min.
Refractive Index: 8X (Attenuation)
Injection Volume: 20 µL

## PREPARATION OF STOCK AND STANDARD SOLUTIONS

### DMSO

DMSO standard solutions were prepared by weighing out separately 15.7, 33.3, 68.5, and 128.1 mg of DMSO in 100-mL volumetric flasks. Reagent grade water was added to the meniscus in each flask to prepare the following standards:

| mg DMSO | CONCENTRATION |
|---|---|
| 15.7 | 157 mg/L |
| 33.3 | 333 mg/L |
| 68.5 | 685 mg/L |
| 128.1 | 1281 mg/L |

### ETHANOL

Ethanol standard solutions were prepared by weighing out separately 105.5, 215.4, 435.2 and 848.5 mg of ethanol in 100-mL volumetric flasks. Reagent grade water was added to the meniscus in each flask to prepare the following standards:

| mg ETHANOL | CONCENTRATION |
|---|---|
| 105.5 | 1055 mg/L |
| 215.4 | 2154 mg/L |
| 435.2 | 4352 mg/L |
| 848.5 | 8485 mg/L |

### FORMAMIDE

Formamide standard solutions were prepared by weighing out separately 11.0, 22.3, 43.6 and 123.6 mg of formamide in 100 mL volumetric flasks. Reagent grade water was added to the meniscus in each flask to prepare the following standards:

| mg FORMAMIDE | CONCENTRATION |
|---|---|
| 11.0 | 110 mg/L |
| 22.3 | 223 mg/L |
| 43.6 | 436 mg/L |
| 123.6 | 1236 mg/L |

### GLYCEROL/FORMAL

Glycerol/Formal standard solutions were prepared by weighing out separately 123.5, 233.2, 423.7 and 847.6 mg of glycerol/formal in 100 mL volumetric flasks. Reagent grade water was added to the meniscus in each flask to prepare the following standards:

| mg GLYCEROL/FORMAL | CONCENTRATION |
|---|---|
| 123.5 | 1235 mg/L |
| 233.2 | 2332 mg/L |
| 423.7 | 4237 mg/L |
| 847.6 | 8476 mg/L |

## SAMPLE PREPARATION AND HANDLING

Each aqueous sample containing an excess concentration of the appropriate compound was diluted with reagent grade water to obtain a sample concentration within the upper and lower limits of the standard curve. No further sample preparation was required; each sample and standard was injected a minimum of four times.

## RESULTS

Figures 1-4 show HPLC chromatograms obtained from aqueous samples of DMSO, ethanol, formamide and glycerol/formal respectively. Each sample peak is symmetrical and well separated from other peaks in each chromatogram.

Calibration curves were constructed by plotting peak areas for all working standards against their concentration. Standard curve data are presented in Table 1. Precision for all four HPLC methods was determined by injecting a low and high concentration sample four times on three separate days. The mean, standard deviation and relative standard deviation are given in Tables 2 and 3.

In order to evaluate method accuracy, recovery studies were conducted on aqueous samples spiked with each compound separately. This was accomplished by taking sample aliquots at two concentration levels and adding a known volume of the appropriate standard. Each aliquot was then analyzed four times to obtain a mean, standard deviation, relative standard deviation and percent recovery (Tables 4 and 5).

## DISCUSSION

High performance liquid chromatography (HPLC) was the analytical method of choice for analyzing DMSO, ethanol, formamide and glycerol/formal because it requires almost no sample preparation and the aqueous samples can be injected directly. Since ethanol and glycerol/formal do not exhibit absorbance of ultraviolet (UV) light, a refractive index detector was used in place of the UV detector. The detectors that the HPLC employs (UV and refractive index) can be easily interchanged or connected in series for the appropriate analytical method. The chromatographic run times were optimized to be short in duration because the aqueous samples were clean and free of any interfering peaks. Since high concentrations of solutes were used in the tests (about 1000-7000 mg/L), our lower detection limits could be extended downward by increasing the detector sensitivity if necessary for trace analysis. Calibration curve (DMSO) data for day one shows a slope that is unusually high when compared to days two and three. The calibration curve for day one was replotted to check for any errors in calculations. Both the original and replotted calibration curve data for day one gave the same value for the slope.

## CONCLUSION

Relatively rapid and reliable HPLC methods have been developed for the determination of dimethyl sulfoxide, ethanol, formamide and glycerol/formal in aqueous samples. Direct injection of the clean aqueous samples eliminates the need for tedious sample preparation. If needed, the methods will allow for more sensitive detection limits. The calibration curves for all four compounds were linear over the concentration ranges analyzed. Precision and accuracy data for all four HPLC methods are presented in the report.

## LITERATURE CITED

1. Ivey, J.P. and P.R. Haddad. 1987. Determination of dimethyl-sulphoxide using ion-exchange chromatography with ultraviolet absorption detection. J. Chromatogr. 391: 309-314.

2. Iwinski, G. and D.R. Jenke. 1987. Determination of alcohols in pharmaceuticals by ion-exclusion chromatography. J. Chromatogr. 392: 397-405.

3. Walbroehl, Y. and J.W. Jorgenson. 1986. Capillary zone electrophoresis of neutral organic molecules by solvophobic association with tetraalkylammonium ion. Anal. Chem. 58:479-481.

4. Registry of Toxic Effects of Chemical Substances. 1985. U.S. Department of Health, Education and Welfare Public Health Service, Center of Disease Control, National Institute for Occupational Safety and Health. Vol. I and II.

## DISTRIBUTION LIST

4 Commander
U.S. Army Medical Research and Development Command
ATTN: SGRD-RMI-S
Fort Detrick
Frederick, MD 21702-5012

2 Commander
U.S. Army Biomedical Research and Development Laboratory
ATTN: SGRD-UBZ-IL
Fort Detrick
Frederick, MD 21702-5010

2 Defense Technical Information Center (DTIC)
ATTN: DTIC-FDAC
Cameron Station
Alexandria, VA 22304-6145

1 Commander
U.S. Army Biomedical Research and Development Laboratory
ATTN: SGRD-UBZ-P
Fort Detrick
Frederick, MD 21702-5010

2 Commandant
Academy of Health Sciences, U.S. Army
ATTN: HSHA-DCD
Fort Sam Houston, TX 78234-6100

TABLE 1. STANDARD CURVE DATA

DMSO

| DAY | SLOPE | R2 VALUE | Y-INTERCEPT |
|---|---|---|---|
| 1 | 25,071 | 1.0000 | 4,984 |
| 2 | 16,175 | 1.0000 | 4,999 |
| 3 | 16,394 | 1.0000 | 4,976 |

ETHANOL

| DAY | SLOPE | R2 VALUE | Y-INTERCEPT |
|---|---|---|---|
| 1 | 11,854 | 0.9996 | 625,627 |
| 2 | 11,988 | 0.9998 | 30,312 |
| 3 | 11,634 | 1.0000 | 195,572 |

FORMAMIDE

| DAY | SLOPE | R2 VALUE | Y-INTERCEPT |
|---|---|---|---|
| 1 | 1,816 | 1.0000 | -2,515 |
| 2 | 1,802 | 1.0000 | -2,305 |
| 3 | 1,794 | 1.0000 | -1,373 |

GLYCEROL/FORMAL

| DAY | SLOPE | R2 VALUE | Y-INTERCEPT |
|---|---|---|---|
| 1 | 18.95 | 0.9957 | -6,560 |
| 2 | 19.25 | 0.9972 | -5,708 |
| 3 | 19.31 | 0.9986 | -6,877 |

TABLE 2. PRECISION DATA FOR DMSO AND ETHANOL

DMSO

LOW LEVEL

| DAY | Mean(mg/L) | S.D. | R.S.D.(%) |
|---|---|---|---|
| 1 | 160 | 0.43 | 0.27 |
| 2 | 159 | 0.24 | 0.15 |
| 3 | 162 | 0.29 | 0.18 |

HIGH LEVEL

| DAY | Mean(mg/L) | S.D. | R.S.D.(%) |
|---|---|---|---|
| 1 | 805 | 5.34 | 0.66 |
| 2 | 725 | 6.64 | 0.92 |
| 3 | 711 | 1.37 | 0.19 |

ETHANOL

LOW LEVEL

| DAY | Mean(mg/L) | S.D. | R.S.D.(%) |
|---|---|---|---|
| 1 | 1,668 | 105.8 | 6.34 |
| 2 | 1,475 | 99.5 | 6.75 |
| 3 | 1,328 | 28.7 | 2.16 |

HIGH LEVEL

| | | | |
|---|---|---|---|
| 1 | 6,301 | 116.3 | 1.85 |
| 2 | 6,409 | 233.9 | 3.65 |
| 3 | 6,299 | 69.0 | 1.09 |

TABLE 3. PRECISION DATA FOR FORMAMIDE AND GLYCEROL/FORMAL

FORMAMIDE

LOW LEVEL

| DAY | Mean(mg/L) | S.D. | R.S.D.(%) |
|---|---|---|---|
| 1 | 113 | 0.50 | 0.44 |
| 2 | 122 | 0.63 | 0.52 |
| 3 | 124 | 0.38 | 0.31 |

HIGH LEVEL

| | | | |
|---|---|---|---|
| 1 | 950 | 3.01 | 0.32 |
| 2 | 1003 | 5.06 | 0.50 |
| 3 | 991 | 3.31 | 0.33 |

GLYCEROL/FORMAL

Low Level

| DAY | Mean(mg/L) | S.D. | R.S.D.(%) |
|---|---|---|---|
| 1 | 1273 | 8.25 | 0.65 |
| 2 | 1213 | 7.06 | 0.58 |
| 3 | 1224 | 13.93 | 1.14 |

High Level

| | | | |
|---|---|---|---|
| 1 | 6209 | 70.50 | 1.14 |
| 2 | 6211 | 23.51 | 0.38 |
| 3 | 6258 | 23.12 | 0.37 |

TABLE 4. ACCURACY DATA FOR DMSO AND ETHANOL

DMSO

LOW LEVEL

| DAY | AMOUNT ADDED (mg/L) | AMOUNT RECOVERED (mg/L) | S.D. | R.S.D. (%) | REC. (%) |
|---|---|---|---|---|---|
| 1 | 167 | 163 | 1.34 | 0.82 | 97.96 |
| 2 | 169 | 171 | 2.27 | 1.33 | 100.86 |
| 3 | 164 | 164 | 0.43 | 0.26 | 100.47 |

HIGH LEVEL

| | | | | | |
|---|---|---|---|---|---|
| 1 | 922 | 947 | 5.28 | 0.56 | 102.74 |
| 2 | 943 | 955 | 2.77 | 0.29 | 101.25 |
| 3 | 931 | 944 | 6.73 | 0.71 | 101.49 |

ETHANOL

LOW LEVEL

| DAY | AMOUNT ADDED (mg/L) | AMOUNT RECOVERED (mg/L) | S.D. | R.S.D. (%) | REC. (%) |
|---|---|---|---|---|---|
| 1 | 1,230 | 1,326 | 68.90 | 5.19 | 107.83 |
| 2 | 1,230 | 1,306 | 36.38 | 2.79 | 106.20 |
| 3 | 1,226 | 1,263 | 63.22 | 5.00 | 103.02 |

HIGH LEVEL

| | | | | | |
|---|---|---|---|---|---|
| 1 | 6,148 | 6,050 | 50.99 | 0.84 | 98.41 |
| 2 | 6,148 | 6,108 | 55.21 | 0.90 | 99.35 |
| 3 | 6,131 | 6,118 | 24.51 | 0.40 | 99.79 |

TABLE 5. ACCURACY DATA FOR FORMAMIDE AND GLYCEROL/FORMAL

FORMANIDE

LOW LEVEL

| DAY | AMOUNT ADDED (mg/L) | AMOUNT RECOVERED (mg/L) | S.D. | R.S.D. (%) | REC. (%) |
|---|---|---|---|---|---|
| 1 | 143 | 142 | 0.37 | 0.26 | 99.29 |
| 2 | 143 | 140 | 1.89 | 1.35 | 98.18 |
| 3 | 143 | 141 | 0.48 | 0.34 | 99.11 |

HIGH LEVEL

| | | | | | |
|---|---|---|---|---|---|
| 1 | 949 | 964 | 4.36 | 0.45 | 101.63 |
| 2 | 949 | 954 | 4.49 | 0.47 | 100.60 |
| 3 | 949 | 948 | 2.35 | 0.25 | 99.95 |

GLYCEROL/FORMAL

LOW LEVEL

| DAY | AMOUNT ADDED (mg/L) | AMOUNT RECOVERED (mg/L) | S.D. | R.S.D. (%) | REC (%) |
|---|---|---|---|---|---|
| 1 | 1331 | 1266 | 42.96 | 3.39 | 95.09 |
| 2 | 1331 | 1201 | 14.59 | 1.21 | 90.25 |
| 3 | 1331 | 1259 | 12.64 | 1.00 | 94.61 |

HIGH LEVEL

| | | | | | |
|---|---|---|---|---|---|
| 1 | 6656 | 6779 | 20.19 | 0.30 | 101.85 |
| 2 | 6656 | 6635 | 12.61 | 0.19 | 99.69 |
| 3 | 6656 | 6705 | 17.91 | 0.27 | 100.73 |