October 3, 2006

SUBJECT: Presence of Azithromycin Sesquihydrate (Form G) in Azithromycin 300 mg, 600 mg, 900 mg and 1200 mg Oral Suspension Samples Manufactured by Pliva Hrvatska d.o.o., Croatia

FROM: Jason Dennis, PhD
Senior Manager/Team Leader
Special Testing & Analytical Development Laboratory

SUMMARY

Commercial samples of azithromycin 300 mg, 600 mg, 900 mg, and 1200 mg oral suspension manufactured by Pliva Hrvatska d.o.o., Croatia were obtained in the United States and forwarded to the Special Testing & Analytical Development Lab at Pfizer, Groton, CT for analysis to determine the form of azithromycin in the product. Samples were analyzed using a combination of ^{13}C solid state nuclear magnetic resonance (ssNMR) spectroscopy, Fourier transform infrared spectroscopy (FTIR), powder X-ray diffraction (PXRD), and gas chromatography. Results from the spectroscopic tests were compared to reference spectra of azithromycin Form G (azithromycin sesquihydrate), Form A (azithromycin dihydrate) and reference spectra of the other forms recorded in US Patent No. 6,977,243.

FTIR analysis of the samples found each to be comprised of primarily sucrose (estimated 85-90%). Further characterization of the IR data for presence of azithromycin Form A was not possible because the bands from sucrose interfered with bands that are diagnostic of Form A. Similarly, the PXRD spectra for each of the samples were comprised primarily of signals from sucrose, however, a few of the most intense bands corresponding to crystalline azithromycin were observed. These bands allowed partial classification of azithromycin as a crystal structure within the Family I isomorphs (Forms F, G, H, J, M, N, O and P) and/or Form A. Absence of detectable signals in the samples for major bands in PXRD spectra of Family II isomorphs (Forms C, D, E and R) and Form Q provided evidence for absence of these crystalline forms in the samples.

Further analysis of the samples by ssNMR and GC provided definitive identification of azithromycin form. The ssNMR spectra for each of the four oral suspension samples demonstrated excellent agreement with the reference ssNMR spectrum of Form G (46 of 49 peaks were detected in samples). The ssNMR spectra of the samples also showed absence of diagnostic signals for Forms A, F, H, J, M, and N. Headspace GC analysis found no detectable n-butanol and n-pentanol in all the samples and supported absence of Forms O and P in the samples. The headspace GC analysis confirmed absence of detectable levels of solvents that are components of azithromycin Forms D, E, J, M, N, and R. Based on the collective results from these analyses, the Pliva oral suspension samples are concluded to contain primarily azithromycin in the sesquihydrate form (Form G).

OBJECTIVES

Testing was performed on Pliva azithromycin 300 mg, 600 mg, 900 mg and 1200 mg oral suspension samples to determine the form of azithromycin present in the sample. A combination of ssNMR, FT-IR and PXRD spectroscopic techniques were used for the analysis. Additional testing by gas chromatography was used to verify presence or absence of solvents associated with various known crystal forms of azithromycin.

SAMPLE DESCRIPTION

The 300 mg, 600 mg, 900 mg and 1200 mg oral suspension samples in a powder form used in these analyses were purchased from AmerisourceBergen and sent directly to Pfizer. Photographs of the 300 mg, 600 mg, 900, mg and 1200 mg samples are presented in **Figure 1**. Package labeling and other details are summarized below.

Manufactured by: Pliva Hrvatska d.o.o., Zagreb, Croatia, for PLIVA®, Inc., East Hanover, NJ 07936
Test sample details: Oral suspension powder in sealed white bottles, Batch No. 232036 (300mg), expiration date 03/2008 Batch No. 307036 (600mg), expiration date 03/2008 Batch No. 353125 (900mg), expiration date 12/2007 Batch No. 201046 (1200mg), expiration date 04/2008.
Claimed active ingredient and strength (from package label): Batch 232036: Each teaspoon (5 mL) contains azithromycin monohydrate equivalent to 100 mg azithromycin (300 mg/bottle) Batch 307036: Each teaspoon (5 mL) contains azithromycin monohydrate equivalent to 200 mg azithromycin (600 mg/bottle) Batch 353125: Each teaspoon (5 mL) contains azithromycin monohydrate equivalent to 200 mg azithromycin (900 mg/bottle) Batch 201046: Each teaspoon (5 mL) contains azithromycin monohydrate equivalent to 200 mg azithromycin (1200 mg/bottle)
Inactive Ingredients (as listed in package insert): Colloidal silicon dioxide, hydroxypropyl cellulose, sucrose, tribasic sodium phosphate, anhydrous, xanthan gum, banana flavor, cherry flavor, vanilla flavor, and FD&C Red No. 40.
Chain of custody: Samples were purchased from AmerisourceBergen, 101 Norfolk Street, Mansfield, MA 02048 and shipped directly to Pfizer's New York Headquarters which then promptly forwarded the samples by Federal Express to the Special Testing & Analytical Development Laboratory at Pfizer SLS, Groton, CT 06340.

ANALYTICAL RESULTS

1. Fourier Transform Infrared Spectroscopy (FTIR) for Azithromycin Dihydrate Determination

A Nicolet model Magna-IR 550 Fourier Infrared (FT-IR) spectrometer was used to analyze the Azithromycin samples (300 mg, 600 mg, 900 mg and 1200 mg). Each sample was analyzed using a potassium bromide (KBr) pellet preparation. Detailed instructions for the analysis may be found in Pfizer Standard Test Procedure (STP) I 3.94. FT-IR analysis of the Azithromycin oral suspension samples as a KBr pellet produced spectra that were consistent with a product that contains primarily sucrose. Content of sucrose in the sample was estimated to be approximately 90% and this amount would easily overwhelm the signals of azithromycin. **Figure 2** shows spectra of

a representative sample (1200 mg) and sucrose and **Figure 3** shows spectra of the sample (1200 mg) and azithromycin dihydrate reference material. Diagnostic IR bands unique for azithromycin dihydrate in the regions of 3560±3 cm^{-1}, 3491±5 cm^{-1}, 1344±3 cm^{-1}, 1282±3 cm^{-1}/1270±3 cm^{-1} (doublet) and 1084±3 cm^{-1} were not found in any strengths of the sample, likely due to presence of high level of sucrose in the product. The detection limit for azithromycin dihydrate is 25% by weight relative to total azithromycin content.

In an effort to search for single azithromycin crystals, a representative sample was manually scanned for azithromycin crystals using a Nicolet model 560 FT-IR spectrometer coupled with a Continuum IR-microscope accessory. None of the selected particles or clusters of particles of the sample showed any enhancement of the diagnostic bands of azithromycin dihydrate (listed above). Thus the FT-IR results from the both techniques are inconclusive as to whether azithromycin dihydrate is present or absent in the Pliva oral suspension samples.

Further analysis was conducted to identify and estimate the quantity of major ingredients using a series of extractions to separate the individual components within each sample. Isolated components were analyzed by FT-IR to determine their identities and weighed to estimate quantity. The 300 mg sample was found to be comprised primarily of the following: azithromycin (estimated 7% w/w), sucrose (estimated 90% w/w) and silica (estimated 2-3% w/w). The 600 mg, 900 mg and 1200 mg samples were found to be comprised primarily of the following: azithromycin (estimated 10% w/w), sucrose (estimated 87% w/w) and silica (estimated 2-3% w/w). These results are consistent with components identified in the package insert (**Figure 1**). Other ingredients listed in the package insert (i.e., hydroxypropylcellulose, tribasic sodium phosphate, xanthan gum, banana flavor, cherry flavor, vanilla flavor and FD&C Red No. 40) were not measured by this technique.

2. Analysis by Powder X-Ray Diffraction (PXRD) for Azithromycin Dihydrate Determination

PXRD diffractograms were collected for the Pliva azithromycin samples using a Bruker D4 X-Ray Diffractometer. A portion of each powder sample was gently ground to a fine powder in a mortar and pestle for the analysis.

The diffractograms obtained from the Pliva 300 mg, 600 mg, 900 mg, and 1200 mg samples are shown in **Figure 4.** All four spectra were essentially identical to each other and all showed an overall spectrum that was most consistent with that of crystalline sucrose. **Figure 5** shows an overlay of the PXRD spectra of sucrose and the 1200 mg dosage strength. Further examination of signals in the 7 to 12 *2-theta* region of the sample shows small, but significant signals at 7.9, 9.8 and 11.3 *2-theta* that correspond to the most intense PXRD signals of Form G azithromycin (**Figure 5**). These three signals are common to both Type A and all azithromycin forms in the Family I isomorphs (Forms F, G, H, J, M, N, O and P). Further analysis by ssNMR was performed to differentiate between the forms.

The absence of PXRD signals in the samples at positions corresponding to the most intense PXRD signals found in the Family II isomorphs (Forms C, D, E, and R) and Form Q provide evidence for absence of these crystalline forms in the Pliva oral suspension samples. **Figure 6** shows an expanded display of the 1200 mg dosage strength. The absence of detectable signals at 3.9, 10.1 and 10.6 *2-theta* in **Figure 6** supports

absence of the Family II isomorphs. Absence of a signal at 6.8 *2-theta* indicates absence of Form Q (hydrate/hemi-tetrahydrofuran solvate) in the samples. The estimated detection limit for azithromycin dihydrate by the PXRD technique is 5% by weight relative to total azithromycin content.

3. Analysis by ^{13}C Solid State NMR (ssNMR)

Under direction of our laboratory, the ^{13}C-ssNMR spectral analyses were conducted at the Pfizer Global Research and Development NMR Laboratory in Groton, CT, USA. Results of these experiments are summarized below. For the analysis, a portion of the sample was ground gently to a powder and a portion of the powder was packed into an NMR tube. A one-dimensional ^{13}C-ssNMR spectrum was collected for each of the samples using a ^{1}H-^{13}C carbon cross-polarization magic angle spinning (CPMAS) technique. Full details of the NMR analyses are reported in research report CP62993_IP06062.17Aug2006.

The resulting ^{13}C CPMAS spectra (ssNMR) of each sample were compared to the spectrum of azithromycin sesquihydrate Form G that had been previously documented (see PharmSci NMR report CP62993.061401, prepared by A. Medek and L. Lohr on May 28, 2002). **Figure 7** shows ssNMR results for the 300 mg, 600 mg, 900 mg and 1200 mg oral suspension samples. Results supported the presence of azithromycin in all strengths as primarily Form G.

Within the Family I isomorphs, each of the other Forms F, H, J, M, N, O, and P may be eliminated by absence of diagnostic ssNMR signals and/or absence of solvents specific to each form. Form F is excluded by absence of the signal for crystal-bound ethanol at 58.0 ppm. Form J is excluded by absence of detectable signals in the sample for crystal-bound n-propanol at 11.5 ppm and 25.2 ppm. Forms M and N are excluded by absence of detectable signals for crystal-bound isopropanol at 26.0 ppm. Form H is not present since its characteristic peaks at 103.2, 82.7, 66.9, 33.3, 15.4 and 7.0 ppm are absent. Headspace GC analysis (discussed in Section 4) demonstrated absence of n-butanol and n-pentanol and provides evidence for excluding the remaining two forms (Forms O and P) in Family I. Azithromycin dihydrate (Type A) is confirmed absent in the samples by lack of characteristic signals at 39.1 ppm, 52.2 ppm and 178.1 ppm.

Table 1 shows the comparison of carbon chemical shifts observed on the sample with those of azithromycin Form G disclosed in the example section of U.S. Patent No. 6,977,243. Of 49 peaks listed in the patent, 46 were identified (within the ±0.2 accuracy limits) in each of the powder samples. Exceptions were noted for peaks at 65.9 ppm, 73.5 ppm, and 83.1 ppm which are found in the Form G reference but are not seen in the sample because they are obscured by signals from other ingredients (likely sucrose). These variations from the Form G reference spectra are to be expected when the material is formulated into a dosage form with other excipients. Signal interferences from crystalline excipients and solid-solid interactions between the azithromycin and excipients materials leading to line width broadening can each contribute to these variations. Overall, the variations are minor and do not preclude a positive identification of Form G in the samples.

4. Analysis by Headspace Gas Chromatography & Gas Chromatography – Mass Spectroscopy (GC-MS).

Analysis for presence of solvents provides additional support to confirm absence of several azithromycin forms in the Pliva azithromycin oral suspensions. Headspace GC analyses were performed using an Agilent 6890 Gas Chromatograph with flame ionization detection. Each sample was dissolved in water (approximately 1000 mg into 50 mL) and then 5 mL aliquots were placed into a 22 mL headspace vial containing 1 g of anhydrous sodium sulfate. Sealed vials were incubated at 85 C for 10 min and then 2 mL of headspace was injected into the chromatograph. Separation was performed using a 30 m x 0.32 mm i.d. RTX-624 (1.8 μm film) capillary column. Oven temperature program was 40 C (5 min hold) – ramp 2 C/min to 90 C (0 min hold) – ramp 30 C/min to 225 C (2 min hold).

The elution sequence and approximate retention times for various solvents on the chromatographic system were established based on a previous study analyzing various aqueous solutions containing solvent reference materials. A summary of solvents and retention times is presented in the table below.

Solvent	Corresponding Azithromycin Form(s)	Retention time
Ethanol	Form F (monohydrate hemi-ethanolate), Form N (water/ethanol/ isopropanolate)	4.2
Isopropanol	Form M (monohydrate/hemi-isopropanolate), Form N (water/ethanol/ isopropanolate)	5.2
Methyl tert-butyl ether	Form R (monohydrate/mono-methyl t-butyl ether solvate)	6.3
n-Propanol	Form J (monohydrate/hemi-n-propanolate)	7.8
Tetrahydrofuran	Form Q (monohydrate/hemi-tetrahydrofuran solvate), Form E (monohydrate mono-tetrahydrofuran solvate)	9.7
Cyclohexane	Form D (monohydrate monocyclohexane solvate)	10.4
n-Butanol	Form O (hemi-hydrate/hemi-n-butanol solvate)	14.6
n-Pentanol	Form P (hemi-hydrate/hemi-n-pentanol solvate)	23.6

None of the above eight solvents were detected in any of the four strengths of Pliva azithromycin oral suspension samples. A representative headspace GC profile obtained for the 900 mg Pliva Azithromycin sample is presented in **Figure 8.** Based on a signal to noise analysis of responses from an external standard solution, limits of detection established previously for n-propanol, isopropanol, 1-butanol, 1-pentanol, and tetrahydrofuran were each found to be 10 ppm (0.001% w/w) or less. The limits of detection for cyclohexane and methyl tert-butyl ether were not specifically measured in the analysis, but would have lower detection limits as a result of relatively lower solubility in water (hence greater concentration in headspace) than the other solvents. The absence of the above eight solvents in the samples supports the ssNMR conclusion that

the Pliva azithromycin samples do not contain detectable amounts of Forms D, E, F, J, M, N, O, P, or R.

In the GC analysis, two significant peaks were detected at the retention times of 19.9 min and 29.4 min (doublet) in all samples (**Figure 8**). These peaks were not associated with any of the above eight solvents. GC-MS analysis by the method discussed below suggested that the unknown peak(s) was isoamyl acetate. Analysis of isoamyl acetate by the headspace GC method revealed that the two peak(s) at retention time (RT) 29.4 min corresponded to isomers of isoamyl acetate and the other peak at RT19.9 min was a related impurity of isoamyl acetate.

GC-MS analyses were performed using an Agilent 5860 Gas Chromatograph equipped with a mass spectroscopy detection using the same sample preparation as that used for Headspace analysis. Sealed vials were incubated at 85 C for 10 min and then 2 mL of headspace was injected into the chromatograph. Separation was performed using a 20 m x 0.18 mm i.d. DB-17 (0.3 μm film) capillary column. Oven temperature program was 38 C (5 min hold) – ramp 5 C/min to 90 C (0 min hold) – ramp 30 C/min to 250 C (0 min hold). The GC-MS analysis confirmed that the major volatile component from the samples was isoamyl acetate. Minor peaks were noted from this analysis including 3-methyl-1-butanol, limonene, 3-methyl butyl butanoate, and 2-methyl butyl butanoate. Isoamyl acetate is likely a component of the flavor used in the azithromycin oral suspension samples (all four strengths). None of the eight solvents associated with the various azithromycin forms was detected in the GC-MS analysis and supports the conclusions from the headspace GC analysis.

CONCLUSIONS

Results from these analyses provide conclusive evidence that the Pliva Azithromycin Oral Suspension 300 mg, 600 mg, 900 mg, and 1200 mg samples tested in this study contained azithromycin as azithromycin sesquihydrate (Form G).

REFERENCES

1. Notebook 108526 p. 53-54
2. Notebook ARG-B-235 p. 146
3. Notebook 2012 pgs. 11ff
4. Pfizer Standard Test Procedure I 3.94 (4/22/98) – Identification of azithromycin dihydrate by Infrared spectroscopy
5. PGRD Report: CP62993_IP06062.17Aug2006, prepared by Tim Hanser
6. PGRD Report CP62993.061401, prepared by A. Medek and L. Lohr on May 28, 2002
6. United States Patent No. 6,977,243 (issued Dec. 20, 2005, Certificate of Correction issued Feb. 7, 2006)

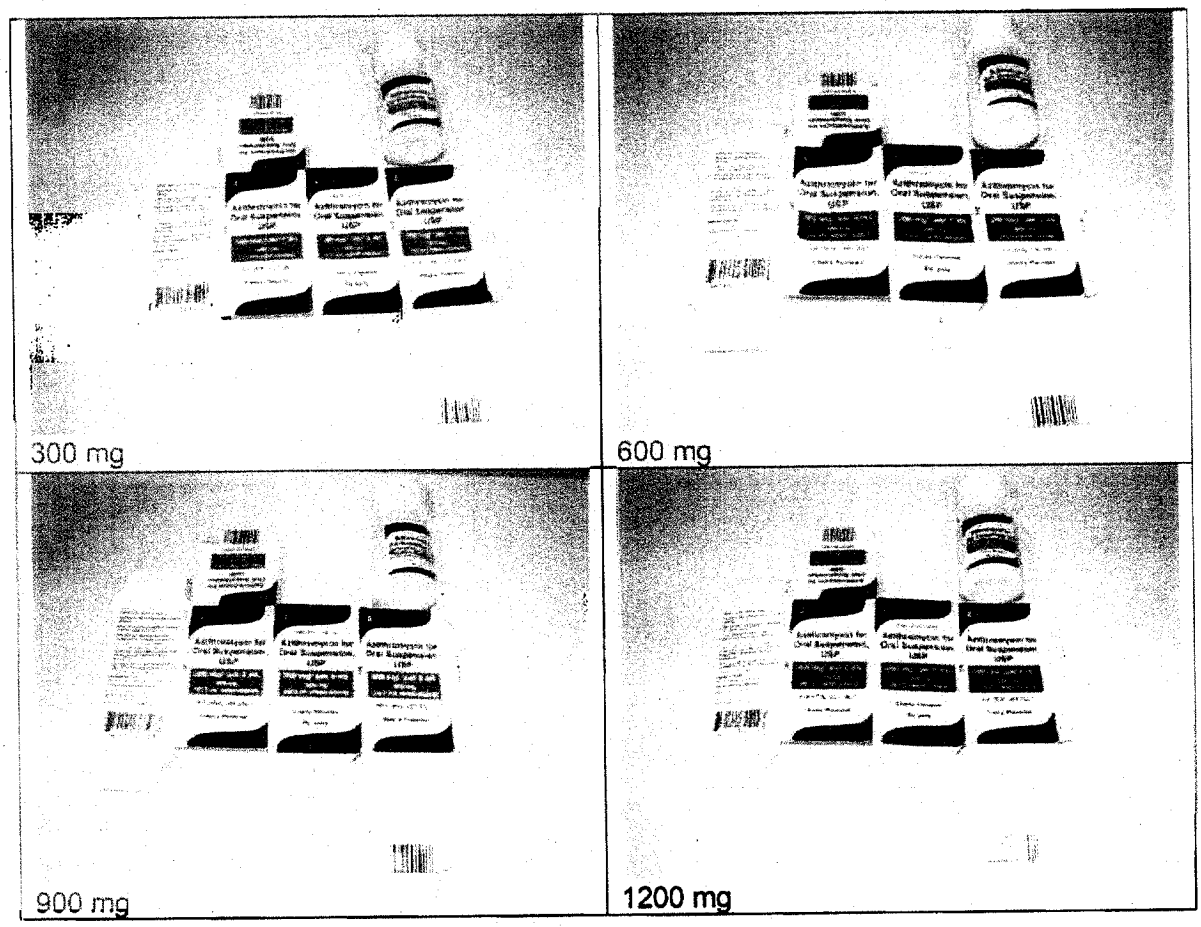


Figure 1. Pliva Azithromycin for Oral Suspension – Photographs of samples received at Pfizer SLS laboratory in Groton.

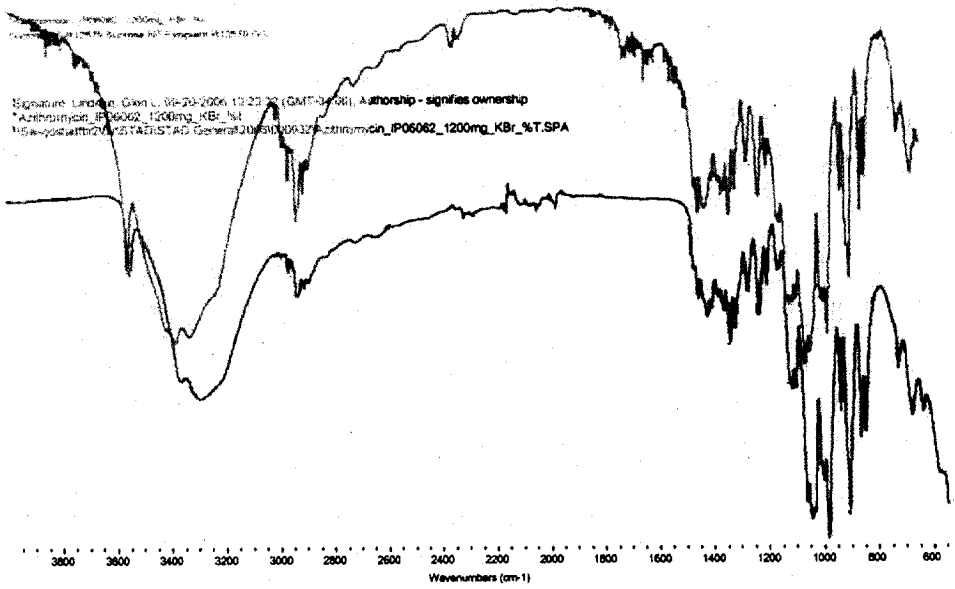
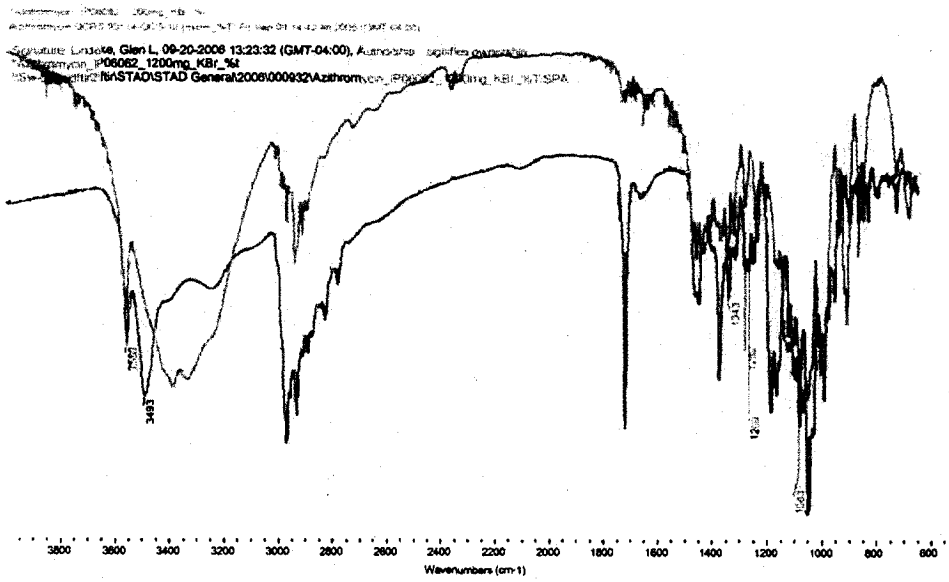


Figure 2. Representative FT-IR spectra of Pliva azithromycin oral suspension (1200 mg) sample (upper trace, red) and Sucrose #R12879 (lower trace, blue). FT-IR analysis revealed that the sample FT-IR spectrum is consistent with that of sucrose, indicating a high content of sucrose present in the sample.

A



B

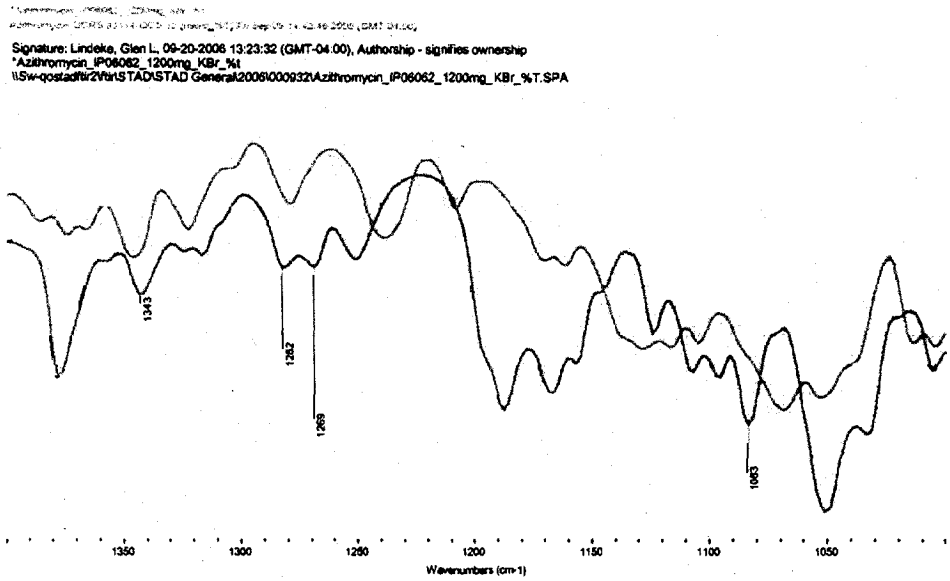


Figure 3. Representative FT-IR spectra of Pliva azithromycin oral suspension (1200 mg) sample (upper trace, red) and azithromycin dihydrate lot G37060-64140-980-1 (lower trace, blue) shown: (**A**) full scale from 4000 cm^{-1} – 400 cm^{-1} and (**B**) expanded scale from 1400 cm^{-1} – 1000 cm^{-1} range. Presence of interfering bands from sucrose prevented determination of presence or absence of azithromycin dihydrate signals in region of 3500 cm^{-1} – 1050 cm^{-1}.

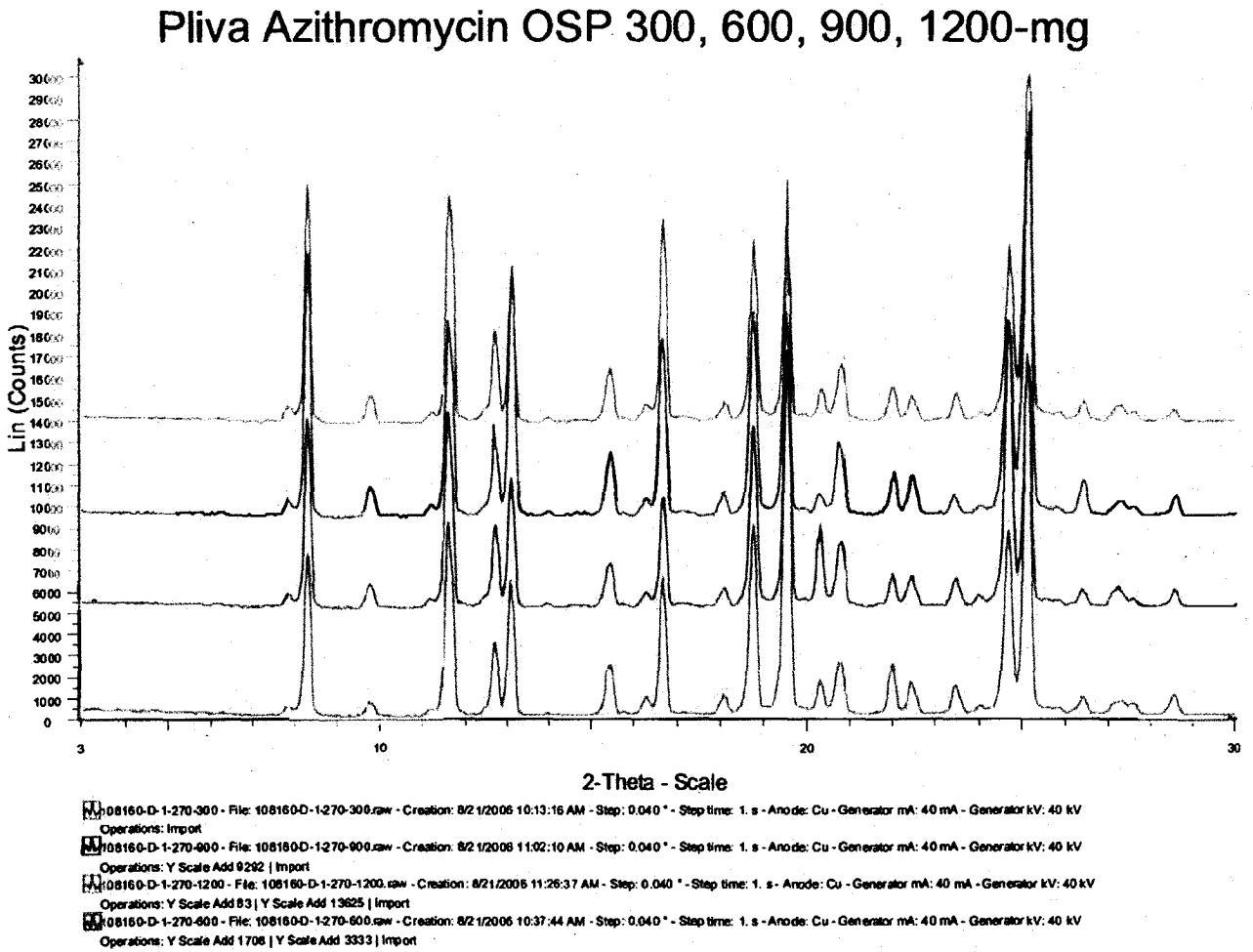


Figure 4. Overlay of diffractograms (2-30 *2-theta*) for Pliva azithromycin 300 mg, 600 mg, 900 mg and 1200 mg oral suspension products. All four strengths show similar profiles, indicating essentially identical composition. Majority of peaks are due to sucrose, the major ingredient in all four formulations.

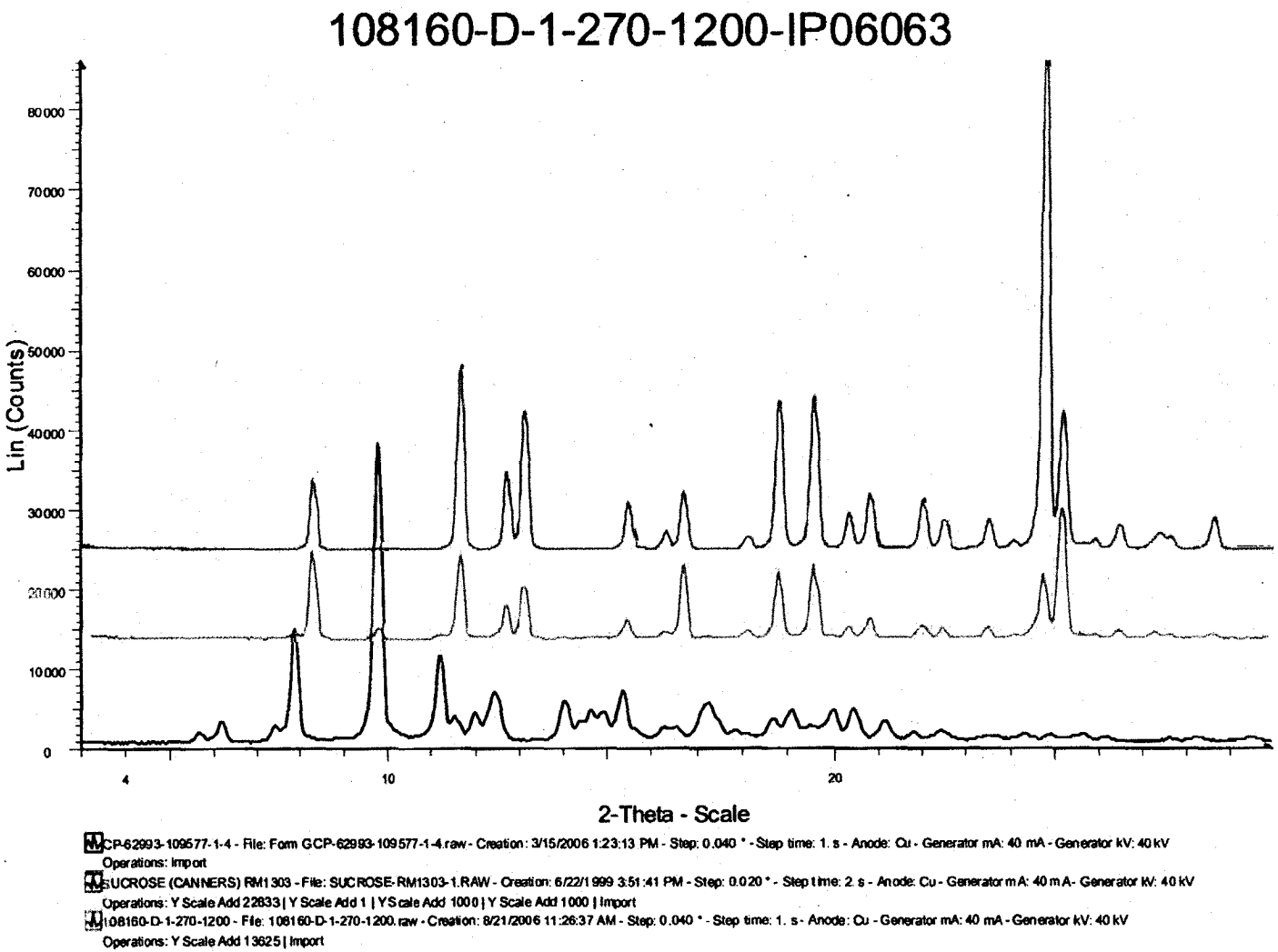


Figure 5. Overlay of diffractograms (2-30 *2-theta*) for Sucrose (top), and Pliva Azithromycin 1200 mg oral suspension (middle), and Azithromycin Form G (bottom). The sample diffractogram shows signals at 7.9, 9.8, and 11.2 *2-theta* that are diagnostic of Form G and other Family I azithromycin isomorphs.

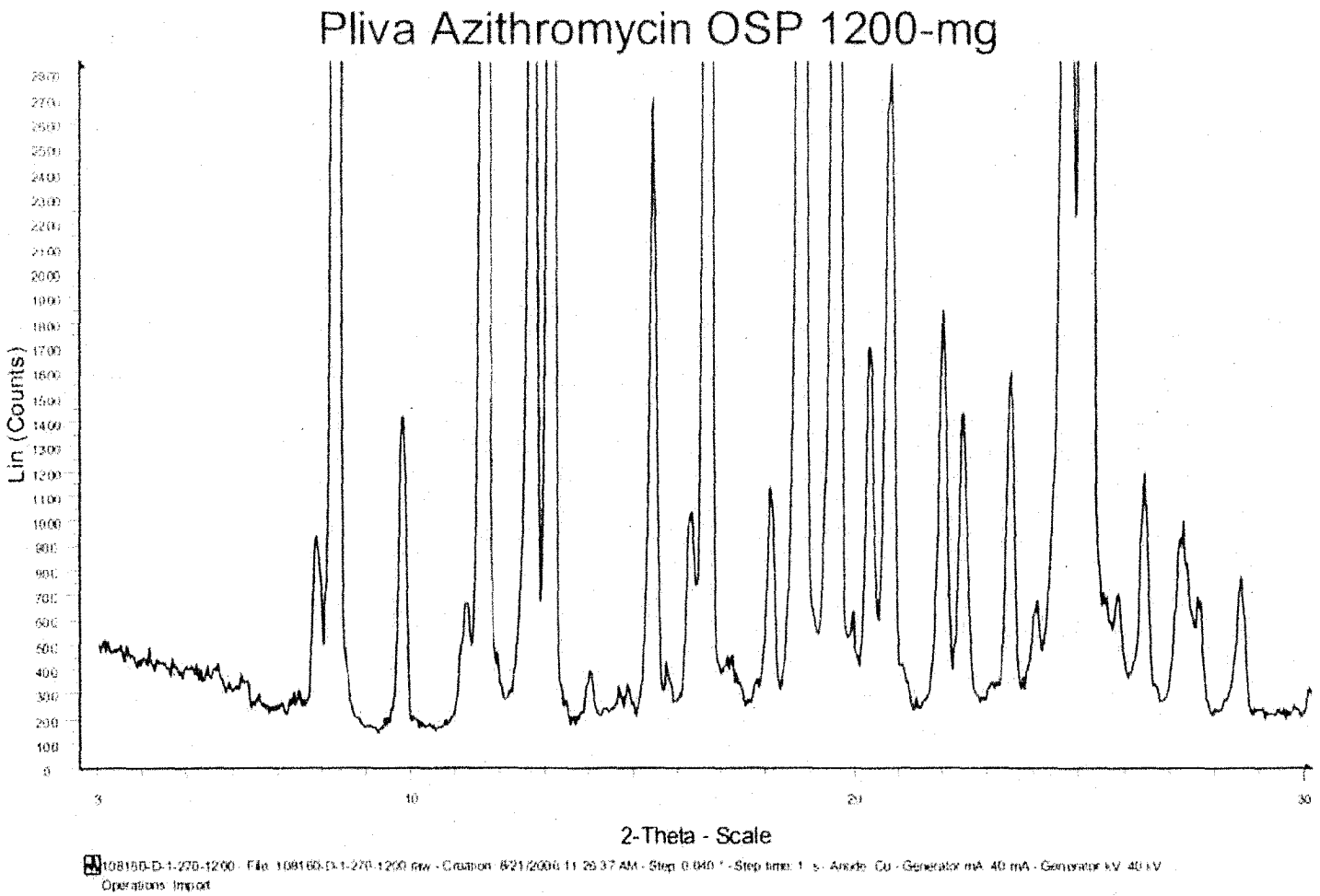


Figure 6. Expanded diffractogram (2-30 *2-Theta*) of Pliva azithromycin 1200 mg oral suspension product to show absence of detectable diagnostic signals for Family II isomorphs (i.e. 3.9, 10.1 and 10.6 *2-theta*) and Form Q (6.8 *2-theta*).

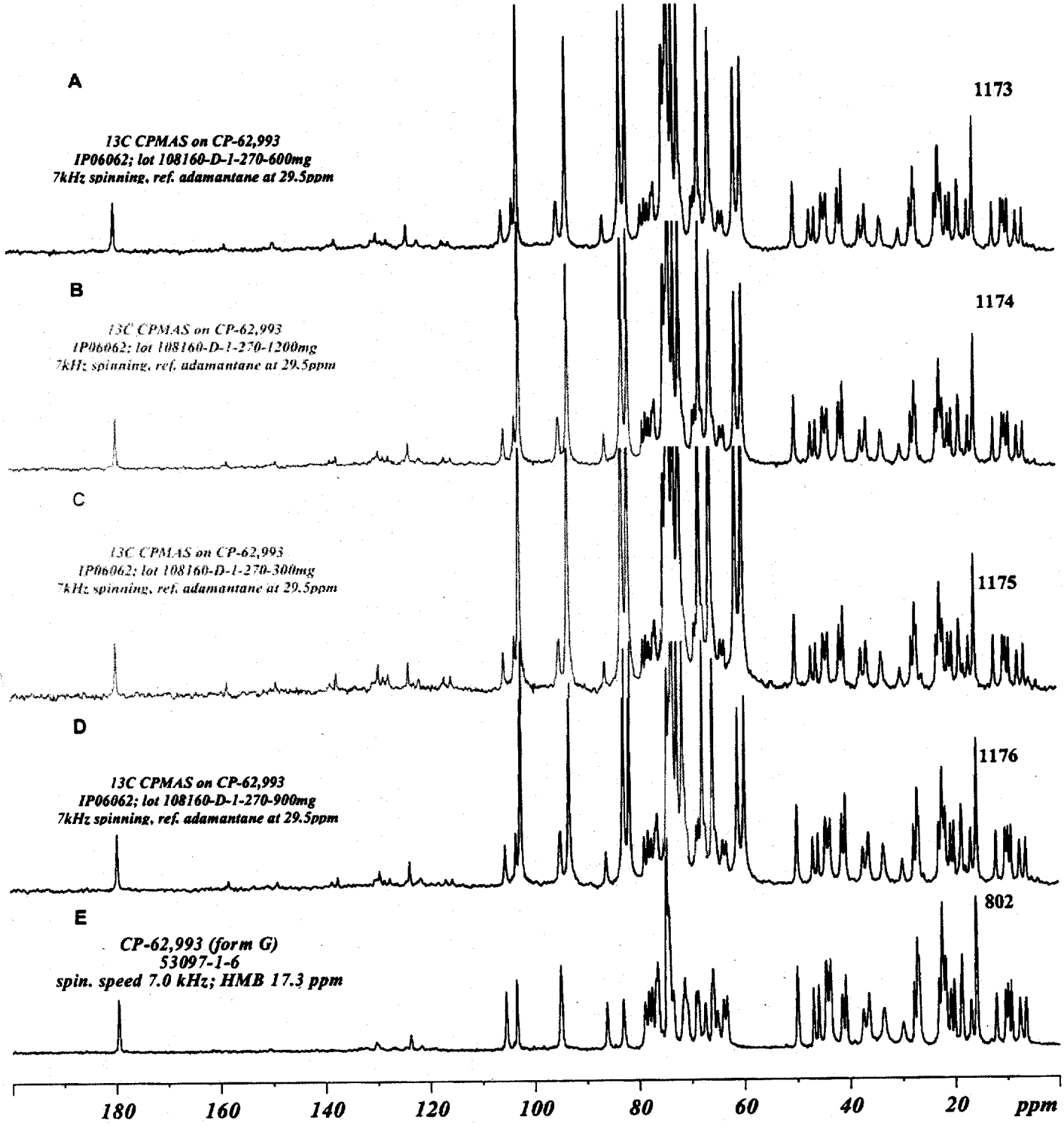


Figure 7. Comparison of ^{13}C CPMAS ssNMR spectra of Pliva oral suspension samples **(A)** 600 mg, **(B)** 1200 mg, **(C)** 300 mg, **(D)** 900 mg, and **(E)** azithromycin sesquihydrate (Form G) reference spectrum?. The sample was found to contain azithromycin sesquihydrate. A detailed comparison of the carbon chemical shifts in ppm units for azithromycin Form G (from U.S. Patent No. 6,977,243) and corresponding azithromycin shifts for the samples is shown in **Table 1**.

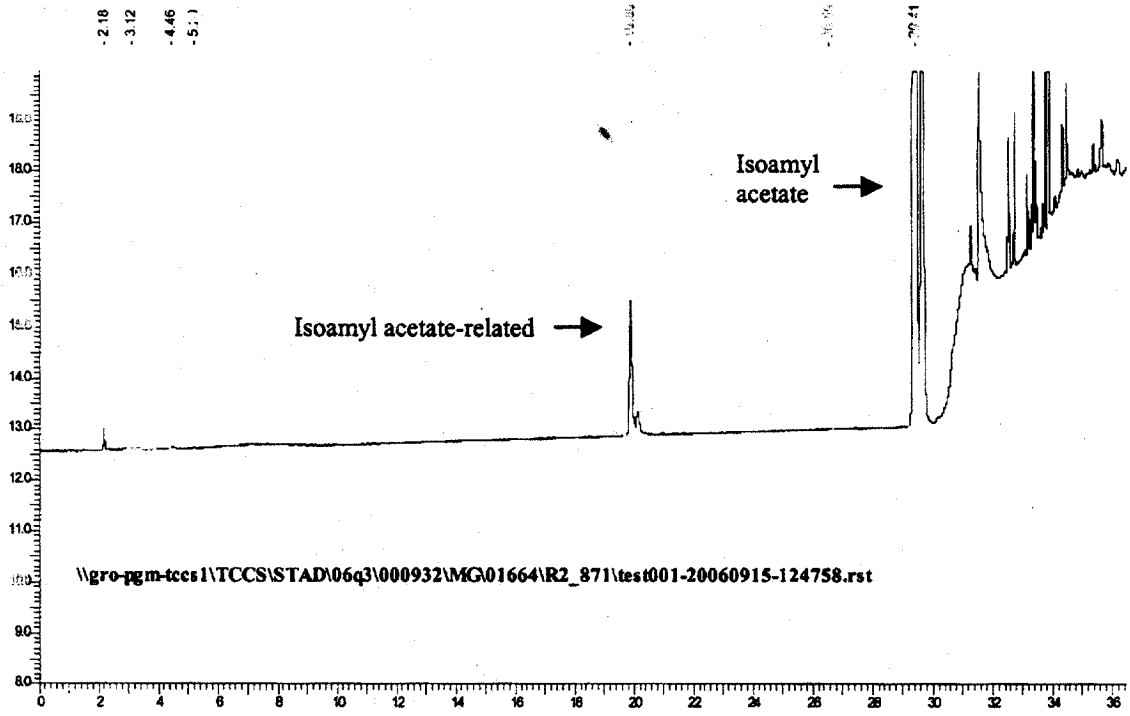


Figure 8. Headspace GC profile for Pliva Azithromycin 900 mg sample. The chromatogram showed presence of isoamyl acetate and its derivative with retention times at 29.4 and 19.9 min, respectively. None of the eight solvents associated with azithromycin crystalline forms were detected in the expected region between 4 to 24 minutes. Peaks eluting after 30 minutes are likely from high boiling flavor-related components and do not impact the conclusions from the headspace GC analysis.

Table 1. ^{13}Carbon ssNMR shifts of azithromycin as listed for Form G in U.S. 6,977,243 and shifts observed in spectra of the Pliva azithromycin samples shown in **Figure 7**. Of 49 peaks listed in the patent, 46 were identified (within the ±0.2 accuracy limits) in each of the tablet samples. Exceptions (noted in footnotes below) were minor and do not preclude a positive identification of Form G in the samples.

300 mg sample (ppm)	600 mg sample (ppm)	900 mg sample (ppm)	1200 mg sample (ppm)	Form G from Patent (accurate within ±0.2 ppm)
179.4	179.5	179.4	179.4	179.5
105.5	105.5	105.5	105.4	105.5
103.4	103.4	103.4	103.4	103.5
95.0	95.0	94.8	94.8	95.0
86.1	86.1	86.1	86.1	86.2
a	A	a	a	83.1
78.8	78.8	78.8	78.8	78.9
78.1	**78.1**	78.1	78.1	78.2
77.5	**77.5**	77.5	77.5	77.6
76.3	**76.7**	76.3	76.3	76.4
75.8	**75.6**	75.6	75.6	75.7
74.6	74.6	74.6	74.6	74.7
74.1	74.1	74.1	74.1	74.3
a	A	a	a	73.5
71.3	71.2	71.2	71.2	71.3
69.0	69.1	69.1	69.0	69.1
68.6	68.6	68.6	68.6	68.8
67.4	67.4	67.4	67.4	67.4
a	A	a	a	65.9
65.2	65.1	65.3	65.3	65.2
63.9	63.9	63.9	63.9	64.0
63.2	63.2	63.2	63.2	63.3
49.9	49.9	49.9	49.9	50.0
46.9	46.9	46.9	46.9	46.9
45.9	45.9	45.9	45.9	46.0
44.5	44.5	44.5	44.5	44.5
43.6	**43.8**	**43.6**	**43.6**	43.7
41.4	**41.4**	41.4	41.4	41.5
40.7	40.7	40.7	40.8	40.8
37.4	37.4	37.4	37.4	37.5
36.4	36.4	36.4	36.4	36.5
33.6	33.6	33.6	33.6	33.6
30.0	30.0	30.0	29.9	30.0
27.8	27.8	27.8	27.8	27.9
27.2	27.2	27.2	27.2	27.3
23.1	23.1	23.1	23.1	23.1
22.4	22.4	22.4	22.4	22.5
21.8	21.8	21.8	21.8	21.9
20.8	20.8	20.8	20.8	20.9
20.1	20.1	20.1	20.1	20.2
18.8	18.8	18.8	18.8	18.8
16.9	16.9	16.9	16.9	17.0
15.9	15.9	15.9	15.9	16.0
12.1	12.1	12.1	12.1	12.2
10.3	10.3	10.3	10.3	10.4
9.8	9.8	**9.8**	**9.8**	**9.9**
9.2	9.2	**9.2**	**9.2**	**9.3**
7.6	7.6	**7.6**	**7.6**	**7.6**
6.4	6.4	**6.4**	**6.4**	**6.5**

(a) Low intensity peak in the standard of Form G. Most likely overlapped with the excipients signals in the sample analysis