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False Carbamazepine Positives Due To 10,11-Dihydro-10-Hydroxycarbamazepine Breakdown in the GC/MS Injector Port

Robert D. Johnson
Russell J. Lewis
Mike K. Angier

Civil Aerospace Medical Institute
Federal Aviation Administration
Oklahoma City, OK 73125

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16. Abstract During the investigation of aviation accidents, postmortem specimens from accident victims are submitted to the Federal Aviation Administration's Civil Aerospace Medical Institute (CAMI) for toxicological analysis. A case recently received by CAMI screened positive for the anticonvulsant medication carbamazepine (Tegretol [®]) by gas chromatography/mass spectrometry (GC/MS). The carbamazepine found during the routine screening procedure was subsequently confirmed using a carbamazepine-specific GC/MS procedure. Concurrently, it was discovered that the accident victim had been prescribed oxcarbazepine (Trileptal [®]). Oxcarbazepine is nearly structurally identical to carbamazepine and is metabolized by cytosolic enzymes in the liver to the active compound 10,11-dihydro-10-hydroxycarbamazepine. The carbamazepine initially found in this case was present due to the breakdown of the active oxcarbazepine metabolite in the GC/MS injector port. In the current study this conversion is investigated, the percentage of carbamazepine formed at various injector port temperatures is determined, and these three compounds are quantified in nine fluid and tissue specimens from the case in question. Lastly, liquid chromatography/mass spectrometry (LC/MS) was used to demonstrate the absence of carbamazepine, and its formation, in the same specimens.					
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FALSE CARBAMAZEPINE POSITIVES DUE TO 10,11-DIHYDRO-10-HYDROXYCARBAMAZEPINE BREAKDOWN IN THE GC/MS INJECTOR PORT

INTRODUCTION

The Federal Aviation Administration's Civil Aerospace Medical Institute (CAMI) is responsible under U.S. Department of Transportation Orders 8020.11B and 1100.2C to "conduct toxicological analysis on specimens from ... aircraft accident fatalities" and "investigate ... general aviation and air carrier accidents and search for biomedical and clinical causes of the accidents, including evidence of ... chemical [use]." Therefore, following an aviation accident, samples are collected at autopsy and sent to CAMI's Forensic Toxicology Research Laboratory, where toxicological analysis is conducted on various postmortem fluids and tissues.

Anticonvulsant medications are commonly prescribed for the treatment of epilepsy.¹⁻³ These medications also act as mood stabilizers and are prescribed in that function for the treatment of bipolar disorder.⁴ Two of the most common anticonvulsant medications are carbamazepine (CBZ, (*Z*)-5*H*-dibenzo[*b,f*]azepine-5-carboxamide, Tegretol[®]) and oxcarbazepine (OXCZ, 10,11-dihydro-10-oxo-5*H*-dibenz[*b,f*]azepine-5-carboxamide, Trileptal[®]). OXCZ, currently one of the top-100 prescribed drugs in America,⁵ is metabolized in the liver, via cytosolic enzymes, to the active metabolite 10,11-dihydro-10-hydroxycarbamazepine (DiCBZ).⁶ These three compounds are nearly identical structurally, as can be seen in Figure 1.

One aviation accident victim recently received by CAMI screened positive for CBZ by gas chromatography/mass spectrometry (GC/MS). The CBZ found during the routine screening procedure was subsequently confirmed and quantified using a CBZ-specific GC/MS procedure.

However, during the investigation it was discovered that the accident victim had been prescribed OXCZ. Specimens from the victim were reanalyzed by GC/MS to confirm and quantify both OXCZ and its metabolite, DiCBZ. While analyzing analytical standards of these compounds by full-scan GC/MS prior to analysis, it became evident that CBZ was present following separate injections of each individual standard.

Numerous liquid chromatography (LC) methods have been developed for the analysis of these anticonvulsant medications. Many of these use mass spectrometry as the detector (LC/MS),^{1,7-11} while others employ ultraviolet (UV) detection.^{12,13} There are, however, few GC/MS methods cited for this purpose. The current study was initiated with the goal of evaluating CBZ production (and subsequent false-CBZ positives) following the GC/MS injection of OXCZ and DiCBZ. This work employed both GC/MS and LC/MS on each specimen analyzed to compare the two analytical techniques.

MATERIALS AND METHODS

Reagents, Standards, and Supplies

All aqueous solutions were prepared using double deionized water (DDW), which was obtained using an ELGA, PURELAB Ultra water system (ELGA, Lowell, MA). All chemicals were purchased in the highest possible purity and used without any further purification. All solvents were of HPLC-grade and were obtained from Fisher Scientific (Fisher Scientific Co., Fair Lawn, NJ). Formic acid (97%) was purchased from ICN (ICN Biomedicals, Inc., Irvine, CA). CBZ was obtained from Cerilliant (Cerilliant Corp., Round Rock, TX), OXCZ

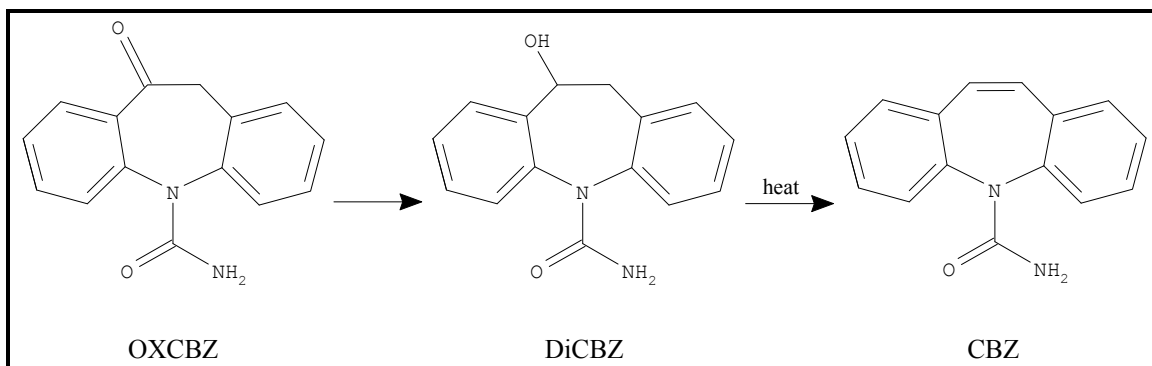


Figure 1. OXCZ metabolizes to DiCBZ, which can then form CBZ, through dehydration, when exposed to high temperatures.

and DiCBZ were obtained from Alltech (Grace Corp., Deerfield, IL), and dihydrocarbamazepine was obtained from Sigma (Sigma-Aldrich Corp., St. Louis, MO).

Stock standard solutions of OXCZB and DiCBZ were prepared at a concentration of 1 mg/mL in methanol. The CBZ standard came at a concentration of 1 mg/mL in a sealed ampoule in methanol. A stock solution of the internal standard (dihydrocarbamazepine) was prepared at a concentration of 100 µg/mL in methanol.

Instrumentation

Gas Chromatographic/Mass Spectroscopic Conditions

The bench-top GC/MS system consisted of a Hewlett Packard (HP) 6890 series GC, interfaced with a HP 5973 quadrupole MS (Agilent, Palo Alto, CA). The GC/MS was operated with a transfer line temperature of 280°C and a source temperature of 250°C. The MS was tuned on a daily basis using perfluorotributylamine. The electron multiplier voltage was set at 106 eV above the tune voltage. Chromatographic separation was achieved using a Varian, FactorFour, crosslinked 100% methyl siloxane capillary column (12 m x 0.2 mm i.d., 0.33 µm film thickness). Helium was employed as the carrier gas and used at a flow rate of 1.0 mL/min. A HP 6890 autosampler was used to inject 1 µL of extract into the GC/MS. The GC was equipped with a split/splitless injection port operated at 250°C in the splitless mode with a purge time of 0.5 min. The oven temperature profile was established as follows: 130 - 290°C at 25°C/min with a final hold time of 1.00 min, resulting in a total run time of 7.40 min. Initially, standards of each compound (1 µL of a 100 ng/µL solution) were injected individually and analyzed using the full-scan mode of the GC/MS, which scanned from 50 to 600 AMU. Quantification and qualifier ions for each analyte were then selected based on both abundance and mass-to-charge ratio (m/z). To increase reproducibility and reduce interference, high mass ions were selected when possible. The ions chosen for each respective analyte and the retention times observed can be seen in Table 1. Upon

selection of unique ions, the MS was run in selected ion monitoring (SIM) mode with a dwell time of 30 msec for each recorded ion.

Liquid Chromatographic/Mass Spectroscopic Conditions

Analyte separation was achieved using a Agilent 1200 series HPLC (Agilent Corp., Wilmington, DE) equipped with a Security Guard™ C-8 guard column (4.0 x 3.0 mm i.d., 3 µm particles) from Phenomenex® (Torrance, CA), followed immediately by an Atlantis™ LC-18 (150 x 4.6 mm i.d., 3 µm particles) analytical column (Waters Corp., Milford, MA). Samples were injected using an Agilent G1329A autosampler (Agilent Corp., Wilmington, DE). Identification and quantification were accomplished using a Thermofisher Scientific model LTQ XL atmospheric pressure chemical ionization (APCI) linear ion trap mass spectrometer (Thermofisher Scientific Corp., San Jose, CA), which utilized nitrogen as the sheath and auxiliary gas. Control of the HPLC system, integration of the chromatographic peaks, and communication with the mass spectrometer were accomplished via a personal computer using Xcalibur™ LC/MS software (Thermofisher Scientific Corp., San Jose, CA).

Calibrators and Controls

Calibration curves were prepared by serial dilution utilizing bovine whole blood as the diluent. Calibrators were prepared from one original stock standard solution of each compound. Controls were prepared in a similar manner as calibrators, using the same bovine whole blood as diluent but employing a second original stock solution. Calibration curves were routinely prepared at a concentration range of 12.5 – 800 ng/mL. A minimum of 5 calibrators were used to construct each calibration curve. Controls used for the validation of each calibration curve constructed were prepared at concentrations of 80, 160, and 320 ng/mL. A dihydrocarbamazepine working internal standard solution was prepared at a final concentration of 400 ng/mL by dilution with DDW from the stock solution.

Table 1. Ions employed and retention times expected for the GC/MS-SIM analysis of CBZ, OXCZB, DiCBZ, and dihydrocarbamazepine.

Compound	Ions (m/z)*	Retention Time
CBZ	193 , 236, 165	4.68
OXCZB	180 , 252, 209	4.95
DiCBZ	193 , 254, 210	5.07
Dihydrocarbamazepine	194 , 238, 180	4.55

* Ions in bold used for quantitation.

Quantification of OXCBZ, DiCBZ, and CBZ in biological specimens was achieved via an internal standard calibration procedure. Response factors were determined for each sample. The response factor was calculated by dividing the area of the analyte peak by the area of the internal standard peak. Calibration curves were prepared by plotting a linear regression of the analyte/internal standard response factor versus the analyte concentration for the calibrators and were used to determine the concentrations of each compound in controls and specimens.

Sample Extraction

Calibrators, controls, and postmortem specimens were prepared and extracted in the following manner. Tissue specimens were homogenized using a PRO250 post-mounted homogenizer (PRO Scientific, Oxford, CT) employing a 30.0 mm saw-toothed generator set to rotate at 22,000 RPM following a 2:1 dilution with 1.00% sodium fluoride (2 g 1.00% NaF:1 g wet tissue). Three mL aliquots of calibrators, controls, and post-mortem fluids and 3 g aliquots of tissue homogenate (1 g tissue) were transferred to individual 15 mL screw-top vials. To each sample, 400 ng of internal standard was added (1.00 mL of the 400 ng/mL stock solution). The samples were vortexed and allowed to stand for 10 min. Six mL of 0.10 M phosphate buffer, pH 6.00 was added to each specimen. The mixture was then placed on a rotary mixing wheel and mixed for 15 min by simple rotation of the wheel at 15 rpm. Centrifugation at 2500×g for 15 min allowed for the removal of cellular debris and proteins. Following centrifugation, the specimens were transferred to Styre Screen solid-phase extraction (SPE) columns (United Chemical Technologies, UCT Inc., Bristol, PA.), which had been pre-conditioned with 2.00 mL methanol, followed by 2.00 mL 0.10 M phosphate buffer, pH 6.00. Care was taken not to dry the SPE column prior to extract addition. Column flow rates of 1 - 2 mL/min were maintained in each step using a Varian 24 port Cerex™ SPE processor (Varian Co., Harbor City, CA.) with a nitrogen pressure of 3 psi. Once each sample had passed through its respective column, the columns were washed with 2 mL 0.10 M phosphate buffer, pH 6.00, and then dried completely with 25 psi nitrogen for 1 min. The columns were then washed by adding 1 mL 1.0 M acetic acid and were then dried completely with 25 psi nitrogen for 2 min. The columns were then washed by adding 2 mL of hexane and were again dried completely with 25 psi nitrogen for 2 min. The analytes were eluted from the columns with 3 mL of methylene chloride. Eluents were evaporated to dryness in a water bath at 40°C under a stream of dry nitrogen, reconstituted in 50 µL ethyl acetate, and transferred to sample

vials for analysis. All specimens were analyzed at one time to avoid inter-assay variations. Specimens with analyte concentrations above the associated calibration curves were diluted by an appropriate factor and re-extracted. When specimen dilution was necessary, a control was diluted by the same factor to ensure dilution accuracy.

RESULTS AND DISCUSSION

Carbamazepine Formation

This study evaluated the formation of CBZ during the analysis of OXCBZ and its metabolite, DiCBZ, at varying injector port temperatures. During the course of the experiment, each standard or specimen analyzed by GC/MS was subsequently evaluated by LC/MS. This additional step was employed to determine whether the source of CBZ formation was the heated GC injector port. Finally, 8 fluid and tissue specimens (blood, liver, lung, kidney, spleen, muscle, brain, and heart) from a fatal aviation accident victim that had previously been found positive for both OXCBZ, DiCBZ, and CBZ were analyzed.

There were no deuterated analogs available for OXCBZ, DiCBZ, or CBZ at the time of this analysis so a structurally related internal standard, dihydrocarbamazepine, was employed. All analytes were extracted using a rapid SPE procedure and assayed by both GC/MS and LC/MS. Even with such a simple extraction procedure, no analyte suffered interference from endogenous matrix components. All analyte peaks were completely resolved by both GC and LC.

OXCBZ and DiCBZ were individually evaluated for possible conversion to CBZ under the extreme temperatures encountered in the GC injection port. The initial observation of the possible conversion of either OXCBZ or DiCBZ to CBZ during the screening process was observed at a GC injector port temp of 250°C. Therefore, the possible conversion of OXCBZ and/or DiCBZ to CBZ in the injector port necessitated the evaluation of the injector port at various temperatures. The GC injector port temperatures evaluated in this study were 200, 225, 250, and 275°C. This evaluation was accomplished by injecting 1 µL of a 100 ng/µL neat standard of either OXCBZ or DiCBZ separately and monitoring the formation of CBZ.

The production of CBZ, monitored by collection of its base peak at *m/z* 193.1, was found to be 0.8 ± 0.1% (n=4), 0.7 ± 0.1% (n=4), 0.9 ± 0.3% (n=4), and 1.1 ± 0.2% (n=4) of the peak area of the OXCBZ base peak at *m/z* 180.1, at GC injection port temperatures of 200, 225, 250, and 275°C, respectively. When analyzed by LC/MS, CBZ was found to be present in the OXCBZ

standard, similar to that found by GC/MS. The lack of GC temperature dependant conversion and the presence of CBZ on LC/MS indicated that there was a trace amount of CBZ contamination in the OXCBZ standard and that no thermal conversion of OXCBZ to CBZ occurred at the temperatures investigated.

The production of CBZ from DiCBZ (base peak at m/z 193.1) was found to be $2.4 \pm 0.3\%$ ($n=4$), $4.8 \pm 0.2\%$ ($n=4$), $12.8 \pm 0.3\%$ ($n=4$), and $26.6 \pm 1.4\%$ ($n=4$) at GC injection port temperatures of 200, 225, 250, and 275°C, respectively. The same DiCBZ standard was analyzed by LC/MS, and CBZ was not detected. The absence of CBZ in the standard analyzed by LC/MS, coupled with the temperature dependant conversion seen on the GC, indicated that there was significant thermal conversion of DiCBZ to CBZ in the heated injector port. The likely mechanism of artifactual CBZ formation is the dehydration of DiCBZ in the heated injector port to form CBZ.

As is clearly evident, the conversion of DiCBZ to CBZ is less of a factor at lower temperatures. However, lower GC inlet temperatures were shown to sacrifice GC/MS sensitivity. Therefore, to minimize the CBZ formation and maximize the analyte response, we chose to use an injector temperature of 225°C for the analysis of all postmortem specimens.

Postmortem Specimen Analysis

In fatal aviation accidents, specimens from accident victims are sent to the Federal Aviation Administration's Forensic Toxicology Research Laboratory for toxicological analysis. During initial drug screening, a recent case appeared to be positive for CBZ and, in fact, was confirmed positive by GC/MS for the presence of CBZ. However, information existed indicating that the pilot was taking OXCBZ, not CBZ. This case was subsequently

re-analyzed and found positive for OXCBZ and DiCBZ, thus prompting this study.

Numerous postmortem fluid and tissue samples from the victim in this case were examined using this optimized GC/MS method to determine the presence of OXCBZ, DiCBZ, and the possible formation of CBZ. All samples found positive for CBZ were also analyzed by LC/MS to determine if the CBZ present was from artifactual formation. Each fluid and tissue specimen available from this victim was analyzed. As previously stated, deuterated analogs of these compounds were not available as internal standards at the time of this study. Therefore, the interpretation of quantitative data in specimen types other than blood should be closely scrutinized due to possible variations in extraction efficiencies between specimen types even though the internal standard chosen was structurally very similar.

OXCBZ, DiCBZ and CBZ were found in all specimens examined by GC/MS, despite the lower inlet temperature employed. The results of the GC/MS analysis are shown in Table 2. OXCBZ was found at concentrations of 0.743, 0.891, 0.973, 2.539, 0.737, 0.391, 0.483, and 0.215 $\mu\text{g/mL}$ ($\mu\text{g/g}$) in blood, liver, lung, kidney, spleen, muscle, brain, and heart specimens, respectively. DiCBZ was found at concentrations of 9.848, 38.741, 25.696, 15.603, 14.676, 26.859, 19.963, and 23.569 $\mu\text{g/mL}$ ($\mu\text{g/g}$) in blood, liver, lung, kidney, spleen, muscle, brain, and heart specimens, respectively. CBZ was found at concentrations of 0.018, 0.068, 0.054, 0.036, detected (below LOQ), 0.036, 0.051, and 0.015 $\mu\text{g/mL}$ ($\mu\text{g/g}$) in blood, liver, lung, kidney, spleen, muscle, brain, and heart specimens, respectively. The CBZ present did not exceed approximately 4% of the DiCBZ peak area in any specimen analyzed, which follows from our earlier findings using neat standards at this GC inlet temperature.

Table 2. Concentrations of OXCBZ, DiCBZ, and CBZ found in fluid and tissue specimens obtained from the victim of a fatal aviation accident.

Specimen	OXCBZ*	DiCBZ*	CBZ*
Blood	0.743	9.848	0.018
Liver	0.891	38.741	0.684
Lung	0.973	25.696	0.536
Kidney	2.539	15.603	0.357
Spleen	0.738	14.676	Detected
Muscle	0.391	26.859	0.036
Brain	0.484	19.963	0.051
Heart	0.215	23.569	0.015

* Concentrations given in the units of $\mu\text{g/mL}$ or $\mu\text{g/g}$.

Concentrations of DiCBZ in the specimens analyzed ranged from 9.8 to 38.7 µg/mL (µg/g). The artifactual formation of CBZ was shown above to be due to the dehydration of DiCBZ in the heated injector port of the GC/MS. Therefore, even under optimal conditions, one would expect to detect CBZ in each of these specimens when analyzed by GC/MS. As expected, this was the case for these specimens. The same specimens were analyzed a second time utilizing LC/MS to determine if CBZ would be detected with this technique. Following analysis it was determined that no CBZ was present in any of the case specimens tested. This further demonstrates the artifactual formation of CBZ when high concentrations of DiCBZ are present in the specimen and the analytical tool employed is GC/MS, regardless of the injector port temperature selected.

CONCLUSION

The findings from this study clearly demonstrate the thermal transformation of the OXCBZ metabolite, DiCBZ, to the structurally related CBZ during analysis. Chemical degradation/transformation is particularly disconcerting in cases resulting in either a false negative or a false positive analytical finding. If the compound formed is readily available and commonly prescribed, the analytical result obtained following analysis would most likely not be questioned. Therefore, it is prudent to be aware that such transformation/degradation can occur during or prior to drug analysis and for a laboratory to maintain a high degree of quality assurance so that such occurrences can be detected if and when they do occur.

REFERENCES

1. Maurer, H.H., Kratzsch, C., Weber, A.A., et al. Validated Assay for Quantification of Oxcarbazepine and Its Active Dihydro Metabolite 10-Hydroxycarbazepine in Plasma by Atmospheric Pressure Chemical Ionization Liquid Chromatography/Mass Spectrometry. *J Mass Spectrom*, 37: 687-92 (2002).
2. Wilson, E.A. and Brodie, M.J. New Antiepileptic Drugs. *Baillieres Clin Neurol*, 5: 723-47 (1996).
3. Bazil, C.W. New Antiepileptic Drugs. *Neurologist*, 8: 71-81 (2002).
4. Juruena, M.F., Ottoni, G.L., Machado-Vieira, R., et al. Bipolar I and II Disorder Residual Symptoms: Oxcarbazepine and Carbamazepine as Add-on Treatment to Lithium in a Double-Blind, Randomized Trial. *Prog Neuropsychopharmacol Biol Psychiatry*, 33: 94-9 (2009).
5. www.Drugs.Com/Top200.Html. (July 2009).
6. Lloyd, P., Flesch, G., and Dieterle, W. Clinical Pharmacology and Pharmacokinetics of Oxcarbazepine. *Epilepsia*, 35 Suppl 3: S10-3 (1994).
7. Breton, H., Cociglio, M., Bressolle, F., et al. Liquid Chromatography-Electrospray Mass Spectrometry Determination of Carbamazepine, Oxcarbazepine and Eight of Their Metabolites in Human Plasma. *J Chromatogr B Analyt Technol Biomed Life Sci*, 828: 80-90 (2005).
8. Corso, G., D'Apolito, O., and Paglia, G. The Accuracy of Oxcarbazepine Quantification by a Liquid Chromatography/Tandem Mass Spectrometry Method Is Influenced by the Ion Source Fragmentation of Its Metabolite Trans-Diol-Carbazepine. *Rapid Commun Mass Spectrom*, 21: 269-72 (2007).
9. de Sousa Maia, M.B., do Nascimento, D.F., Martins, I.L., et al. Simultaneous Quantitative Analysis of Oxcarbazepine and 10,11-Dihydro-10-Hydroxycarbazepine in Human Plasma by Liquid Chromatography-Electrospray Tandem Mass Spectrometry. *J Pharm Biomed Anal*, 45: 304-11 (2007).
10. Klys, M., Rojek, S., and Bolechala, F. Determination of Oxcarbazepine and Its Metabolites in Postmortem Blood and Hair by Means of Liquid Chromatography with Mass Detection (HPLC/APCI/MS). *J Chromatogr B Analyt Technol Biomed Life Sci*, 825: 38-46 (2005).
11. Paglia, G., D'Apolito, O., Garofalo, D., et al. Development and Validation of a LC/MS/MS Method for Simultaneous Quantification of Oxcarbazepine and Its Main Metabolites in Human Serum. *J Chromatogr B Analyt Technol Biomed Life Sci*, 860: 153-9 (2007).
12. Greiner-Sosanko, E., Giannoutsos, S., Lower, D.R., et al. Drug Monitoring: Simultaneous Analysis of Lamotrigine, Oxcarbazepine, 10-Hydroxycarbazepine, and Zonisamide by HPLC-UV and a Rapid GC Method Using a Nitrogen-Phosphorus Detector for Levetiracetam. *J Chromatogr Sci*, 45: 616-22 (2007).
13. Kimiskidis, V., Spanakis, M., Niopas, I., et al. Development and Validation of a High Performance Liquid Chromatographic Method for the Determination of Oxcarbazepine and Its Main Metabolites in Human Plasma and Cerebrospinal Fluid and Its Application to Pharmacokinetic Study. *J Pharm Biomed Anal*, 43: 763-8 (2007).

