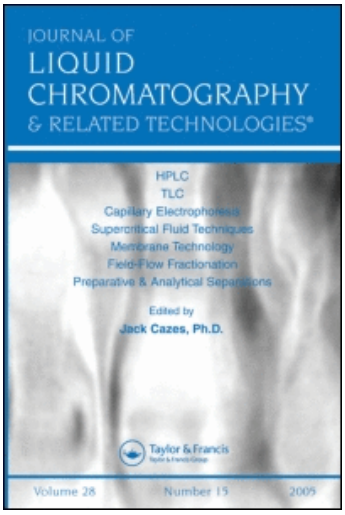

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A SIMPLE AND SENSITIVE APCI-LC-MS METHOD FOR THE DETECTION OF THE ANTITUMOR AGENT, CARMUSTINE (BCNU) IN RAT PLASMA

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□ *A simple and sensitive LC-MS method for the quantitative determination of the antitumor agent, N,N′-bis (2-chloroethyl)-N-nitrosourea (BCNU or carmustine), is described in this report. The method has been developed using an atmospheric pressure chemical ionization (APCI) probe on an ion trap mass spectrometer. A formate adduct ($[M+45]^-$) of BCNU was observed in the negative mode at 257.8 m/z and further confirmation for BCNU was obtained by observing the characteristic isotopic pattern in the mass spectrum, which indicated the compound contained two chlorine atoms. The extraction of BCNU from rat plasma with isopropyl ether:hexane (1:1) resulted in 81.3% of sample recovery. The limit of detection (LOD) calculated for the method was 1.25 ng (injected amount) using a standard solution of BCNU (S/N= 9:1). Optimum linearity and reproducibility data were obtained for BCNU; $r^2 = 0.9999$ (0.2 μg/mL to 10.2 μg/mL) with precision of (RSD) ≤ 6.4% at two different concentrations and accuracy of 92.3 and 102.4%.*

Keywords APCI, BCNU, carmustine, LC-MS, post column formate adduct

INTRODUCTION

Mass spectrometry coupled with high performance liquid chromatography has been a widely used tool for analytical assays for various drugs.[1–3] Advances in LC-MS interface technologies using atmospheric pressure ionization probes, including electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and atmospheric pressure photoionization (APPI) have allowed the development of selective and sensitive methods for targeting drugs.[4] There has been extensive growth in

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applications for LC-MS, and retention time and molecular weight are emerging as essential analytical features necessary to develop a product from a drug target.[1] High throughput analysis of formulations, impurity profiling, as well as bioavailability studies can be carried out more easily with LC-MS methods using automated sample injectors and processing software.[4]

N,N′-bis (2-chloroethyl)-*N*-nitrosourea (BCNU = carmustine) is an FDA approved antineoplastic agent for the treatment of malignant brain tumors and has been a mainstay of chemotherapy treatment for brain tumors for more than twenty years.[5] It decomposes under physiological conditions and one of the decomposition products (alkylating agent) is the proposed active molecule.[6,7] Previously reported analytical methods for the analysis of BCNU include a colorimetric assay,[8] high performance liquid chromatography coupled with a UV detector,[9] chemical ionization mass spectrometry analysis.[10] and gas chromatography coupled with various detectors including mass spectrometry and an electron capture detector.[5,11] These reported methods are either non-selective or require a derivatization treatment, which makes them unsuitable for selective high throughput analysis.

Brain tumors are still considered a major challenge and the average survival period of patients after surgical treatment combined with radiation and chemotherapy is only a matter of a few years.[12–14] Various academic and industrial labs are conducting research on the development of new drugs or development of drug delivery systems based on the existing drugs for brain tumor treatment.[15,16] Gliadel® wafer, a biodegradable hydrophobic copolymer matrix impregnated with BCNU, is an example of one successful FDA approved treatment method.[14,15,17] However, the colorimetric assay, which has still been used in various studies for the quantitation of BCNU, suggested a great need for a reliable, simple, and specific LC-MS method.[14] An ineffective ionization of BCNU, which is one reason that many LC-MS methods fail, might be responsible for the absence of LC-MS methods in the current literature. These observations encouraged us to develop an LC-MS method for the unstable carmustine molecule. Here, we report the first LC-MS method for the analysis of BCNU. This method is sensitive enough for various applications such as analysis of plasma samples for the detection and quantitation of BCNU, bulk drug analysis, and assay of BCNU in the pharmaceutical dosage form.

EXPERIMENTAL

Reagents

BCNU was purchased from Sigma Aldrich, ammonium formate (Fluka grade), acetonitrile (HPLC grade), hexanes, and isopropyl ether were purchased from Caledon and ACROS, and rat plasma was kindly donated by Dr. T. Saleh (UPEI).

Procedures

Standard Solutions

For the standard calibration curve (0.2 μg/mL to 10.2 μg/mL) and lower limit of detection experiments, solutions were prepared in acetonitrile using BCNU powder.

Recovery Experiment – Extraction Procedure

To check the extraction efficiency, BCNU was recovered from rat plasma, which was previously spiked (in vitro) with a known amount of a BCNU standard. This procedure was performed with six replicates with 10.2 μg of the standard. Samples were vortexed with 1 mL of hexanes:isopropyl ether (1:1) and, after approximately 2 mins, the supernatant was transferred into a scintillation vial. This step was repeated 4 times to ensure the extraction was complete. The solvent was evaporated under N_2 flow, reconstituted with 1 mL of acetonitrile and 12.5 μL used for LC-MS analysis.

Chromatographic Separation

The Thermo Scientific Accela LC system was used for injection and chromatography with an Altima C-18 HPLC column, 150 × 4.6 mm, 5 μm particle size column, using an acetonitrile:water gradient system at a flow rate 1 mL/min, and the column was maintained at 30°C using a column oven. The gradient used was: 80% water (hold 5 min.), followed by a linear gradient to 100% acetonitrile over the period of 10 min with a final hold for 5 min at 100% acetonitrile. For the equilibration of the system, the solvent system was brought back to the initial conditions for 5 min resulting in a 25 min run time.

Mass Spectrometry Conditions

The Thermo Scientific LXQ ion trap mass spectrometer with the APCI probe was used in this study. The method details are as follows: Full MS, scan range-100 to 800 m/z, polarity-negative, vaporizer temp 200°C, sheath gas 40 (arb), auxiliary gas 10 (arb), discharge current 5 μA, and capillary temperature 150°C. All of the ion optics voltages were optimized by automatic and semi-automatic tuning. Ammonium formate was teed in at the rate of 0.6 μmoL/min (0.1 μL/min of 6 M solution) by the syringe pump to get a stable signal for the formate adduct of BCNU.

Method Validation

Method validation was performed including linearity and range, lower limit of quantification, system precision, recovery from plasma samples, and method precision. Lower limit of detection (S/N = 9:1) was 1.25 ng of BCNU which was equivalent to a 0.1 μg/mL solution with a 12.5 μL

injection volume. Six replicate injections of the sample with 0.2 μg/mL concentration gave precision of 17.8% (RSD) and accuracy of 114.2% suggested the lower limit of quantitation (S/N = 17:1). Linearity range tested from 0.2 μg/mL to 10.2 μg/mL using 5 different concentration levels revealed $r^2 = 0.9999$. System precision was checked using two concentrations and six replicates: 1.02 ppm and 10.2 ppm, % RSD-6.42% and 5.10%, respectively, with accuracy of 92.3 and 102.4%. Matrix interferences were investigated by recovering BCNU from rat plasma. BCNU (10.2 μg) was added to the rat plasma and 8.29 μg recovered (average of 6 samples, RSD = 10.5%) as calculated, using a calibration curve which represents 81.3% as an average recovery.

RESULTS AND DISCUSSION

BCNU (**1**) is a well known antitumor drug and one of its degradation products has the ability to cross the blood brain barrier, which makes it effective against brain tumors.[5,14,17] Analysis of BCNU has been a challenge because of its instability in solution.[6] As mentioned in the introduction, there are several analytical methods in the literature which either require derivatization or the use of deuterated isotopes. A simple LC-MS method would offer a significant advantage for the day-to-day routine analysis of BCNU. One LC-MS study developed for the detection of BCNU involved the use of atmospheric pressure ionization using an IonSpray interface; however, this did not allow observation of intact BCNU but rather just reactive intermediates.[7] Based on this reference, atmospheric pressure ionization with the electrospray (ESI) interface was used as a starting point for the development of a method for the detection of intact BCNU. With ESI, we did not observe any ions with the expected mass to charge ratio and thus, investigated various parameters such as polarity (positive and negative), temperature, solvents, and buffers to maintain pH. In the positive mode, we observed an ion at 185 m/z which is likely to be the protonated denitroso carmustine (**2**).

In order to try to directly detect BCNU, different probe positions and flow rates with the syringe pump and with the LC pump were explored. Solvents such as acetonitrile (with and without formic acid), methanol, water, ammonium acetate buffer, buffer:acetonitrile (1:1), were tried but with no success. Due to the thermal sensitivity of BCNU, the capillary temperature was altered in the range of 80–250°C but this also did not result in the observation of the expected ions. To try to improve the signal by affecting the desolvation, gases such as sheath, auxiliary, and sweep gas were varied. In the negative ESI mode we observed a weak signal for $[M+Cl]^-$ but were not successful in improving the signal intensity.

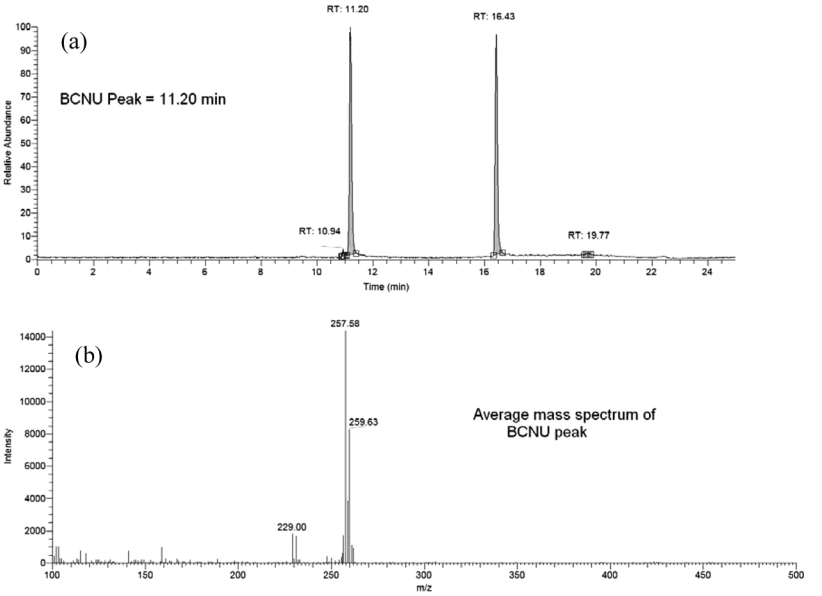

FIGURE 1 (a) Extracted ion chromatogram (XIC) showing BCNU peak at 11.20 min. (peak at 16.43 min is from plasma interference), (b) Mass spectrum for the BCNU peak.

Following these trials with the ESI probe, the atmospheric pressure chemical ionization (APCI) probe was examined. In the positive mode we did not observe the $[M+H]^+$ ion however, the negative mode ionization revealed the presence of a 257.8 m/z ion $[M+45]^-$, a formate adduct. Analysis of the resulting mass spectrum showed the presence of the characteristic isotopic pattern for two chlorine atoms providing further confirmation of the intact BCNU molecule. To increase the signal, formate ions were provided as ammonium formate through the syringe pump post-column immediately before ionization. The MS was tuned with the observed ion (257.8 m/z) followed by optimization of all the parameters discussed above to achieve maximum signal intensity. We speculate that the formate adduct helps to stabilize the BCNU molecule during ionization.

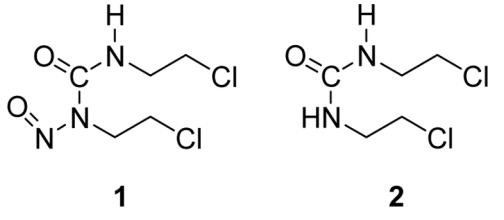

FIGURE 2 Structure of BCNU (carmustine) and denitroso carmustine.

Following successful MS detection, we developed an LC method using rat plasma matrix for the interference. As ammonium formate was necessary for the ionization, it was added to the mobile phase however, this led to the deformation of the BCNU peak into a broad signal. Hence, ammonium formate was mixed with the mobile phase after column elution and just prior to the ionization via the syringe pump. A reversed phase column using an acetonitrile:water gradient mobile phase was optimized to obtain suitable peak shape as well as resolve BCNU from plasma matrix interferences. This chromatographic system allowed the separation of BCNU from a closely eluting compound from rat plasma. A method for the efficient extraction of BCNU from plasma matrix was developed based on reported data.[5,8,10,11] A variety of solvents including acetonitrile, methanol, ethyl acetate/hexane, and hexane/isopropyl ether were evaluated, and the extraction with hexane/isopropyl ether proved to be optimal and led to 81.3% sample recovery. The BCNU solutions were tested in the range of 0.2 to 10.2 μg/mL ($r^2 = 0.9999$) with 0.2 μg/mL as lower limit of quantitation (S/N = 17:1) (RSD = 17.8%, n = 6). Using acetonitrile as a blank solution, 1.25 ng of BCNU was the lowest amount detected with this method (S/N = 9:1, ICIS algorithm was used for calculation). Reproducibility was established by analyzing standard samples at two different concentrations (system precision, RSD ≤6.4% at 1 μg/mL and 10 μg/mL, n = 6 and accuracy of 92.3 and 102.4% and method precision, RSD = 10.5%, n = 6).

CONCLUSION

In summary, an LC-MS method was developed based on the detection of the formate adduct of BCNU using an ion trap mass spectrometer. This method provides a convenient, specific, and sensitive detection of BCNU in plasma samples for a variety of analytical applications.

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REFERENCES

1. Lee, M.S.; Kerns, E.H. Mass Spectrom. Rev. **1999**, *18*, 187.
2. Manini, P.; Andreoli, R.; Niessen, W.M.A. J. Chromatogr. A. **2004**, *1058*, 21.

3. Smyth, W.F.; Rodriguez, V. J. Chromatogr. A. **2007**, *1159*, 159.
4. Ackermann, B.L.; Berna, M.J.; Murphy, A.T. Curr. Topics Med. Chem. **2002**, *2*, 53.
5. Hassenbusch, S.J.; Michael, Colvin, O.; Anderson, J.H. J. Pharm. Sci. **1995**, *84*, 840.
6. Davis, M.R.; Kassahun, K.; Jochheim, C.M.; Brandt, K.M.; Baillie, T.A. Chem. Res. Toxicol. **1993**, *6*, 376.
7. Davis, M.R.; Baillie, T.A. J. Mass Spectrom. **1995**, *30*, 57.
8. Loo, T.; Dion, R.I. J. Pharm. Sci. **1965**, *54*, 809.
9. Yeager, R.L.; Oldfield, E.H.; Chatterji, D.C. J. Chromatogr. **1984**, *305*, 496.
10. Weinkam, R.J.; Wen, J.H.; Furst, D.E.; Levin, V.A. Clin. Chem. **1978**, *24*, 45.
11. Smith, R.G.; Blackstock, S.C.; Cheung, L.K.; Loo, T.L. Anal. Chem. **1981**, *53*, 1205.
12. Legler, J.M.; Ries, L.A.G.; Smith, M.A.; Warren, J.L.; Heineman, E.F.; Kaplan, E.F.; Linet, M.S. J. Natl. Cancer Inst. **1999**, *91*, 1382.
13. Segal, G. J. Natl. Cancer Inst. **2000**, *92*, 77.
14. Kim, G.Y.; Tyler, B.M.; Tupper, M.M.; Karp, J.M.; Langer, R.S.; Brem, H.; Cima, M.J. J. Controlled Release **2007**, *123*, 172.
15. Lesniak, M.S.; Brem, H. Nat. Rev. Drug Discov. **2004**, *3*, 499.
16. Rich, J.N.; Bigner, D.D. Nat. Rev. Drug Discov. **2004**, *3*, 430.
17. Dyke, J.P.; Sanelli, P.C.; Voss, H.U.; Serventi, J.V.; Stieg, P.E.; Schwartz, T.H.; Ballon, D.; Shungu, D.C.; Pannullo, S.C. J. Neurooncol. **2007**, *82*, 103.