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Journal Name:	Journal of Advances in Medical and Pharmaceutical Sciences
Manuscript Number:	2014_JAMPS_12953
Title of the Manuscript:	Analysis of O6-Methylguanine in Cancer Patient Blood during Administration of Cyclophosphamide using Ultra High Performance Liquid Chromatography-Tandem Mass Spectrometry
Type of the Article	Original Research Article

General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

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PART 1: Review Comments

	Reviewer's comment	Author's comment <i>(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)</i>
<u>Compulsory</u> REVISION comments	In its present form this manuscript is very difficult to understand and has several flaws. The writing is poor and it is not possible to exactly know what was done. One of the main reasons for the lack of clarity is that the manuscript needs grammatical revision by a native speaker. The objectives are not clearly stated ("The goals of this research are to analyze and quantify the distribution of O6-methylguanine formed in cancer patients who received it during their chemotherapy." Did the patients receive O6-methylguanine? The authors want to analyze the distribution of O6-methylguanine, in what/where? More important, it appears that the authors were not aware that their method is a bioanalytical one. Indeed, only the ICH guideline Q2 R1 was referenced (ref.17). What about the FDA and EMA guidelines on bioanalytical methods? Did the authors prepare the calibration curve in the same biological matrix as the samples in the intended study by spiking the matrix with known concentrations of the analyte? What about the stability evaluation? The conclusions are strongly affected by the missing data and improper validation of the bioanalytical method.	Noted. This paper has been submitted and corrected by American Journal Expert (AJE), and the certificate from them has been sent by email to JAMPS editor. Noted, corrected and marked in the INTRODUCTION. DNA adduct is resulted from reaction of alkylating agents with DNA inside the patient body. O⁶-methylguanine was acquired from DNA hydrolysis in the form of hydrolysate in formic acid, so not in the biological matrix although the preliminary matrix was blood, thus the validation performed in this study does not follow bioanalytical method validation, therefore referring to ICH guideline.



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<u>Minor</u> REVISION comments		
<u>Optional/General</u> comments	1- Unclear sentences and misspellings (I am indicating only those found in the INTRODUCTION.) "Cyclophosphamide is an antineoplastic alkylating agent group that alkylates DNA at". group should be deleted. "Hodgkin and non-Hodgkin lymphoma"... should be replaced by Hodgkin's and non-Hodgkin's lymphoma (in the introduction and in the entire manuscript). "DNA has several nucleophilic sites that are vulnerable alkylating agents", "DNA adduct O₆-alkylguanine", "breast cancer patient blood"..... 2- Abstract: There are many unclear sentences and misspellings. Also, the statement "1 sample at a concentration of 5.8680 ng/mL" should be revised. The significant figures are too much (four decimals!!) 3- The authors should include references to support the following statement: "Many methods for the analysis of DNA adducts have been reported". 4- The legend of Figure 1 should be: Chemical structures of and 5- Material and methods: This section should be rewritten. It is unclear and many important details were omitted. Did the patients consent to participate in this study? How many mg did the patients receive? What were the inclusion and exclusion criteria? Although the authors used a commercial kit to separate DNA adducts, a brief description of the methodology would be useful. 6- The legend of Tab 1 should be rewritten. Also, why did the authors use commas (e.g. 96,10)? 7- "adenin and guanin" should be replaced by adenine and guanine in the entire manuscript. 8- 3.1. Analysis of the O₆-methylguanine in samples: The characteristics of the patients, doses, drugs, cycles, etc. should be included in a table. Also, the results should be described with tables.	1) Noted, corrected, and marked. 2) Noted, corrected to be 2 decimals, and marked. 3) Noted. 4) Noted, corrected, and marked. 5) Noted, corrected, and marked. 6) Noted, corrected, and marked. 7) Noted, corrected, and marked. 8) Noted and corrected. 9) Noted and corrected.



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	9- Figure 2: Did the authors propose these patters? If not, a suitable reference should be included. 10- 2.3. The UPLC-MS/MS conditions: the authors should uniform the significant figures, i.e. 166.1>149.1 (one decimal) and 152.16>110.28 (two decimals). The mass spectrometer should be described appropriately. 11- Tab 2. The significant figures of the concentration values are not consistent (four decimals???) 12- Why did the authors quantify only O₆-methylguanine? They developed and validated a method for both compounds. Also, they stated "A sensitive and selective analytical method for N₇-alkylguanine and O₆-alkylguanine is needed because those compounds are isomer and are found at low concentrations in the biological samples" 13- Pag 7 is very confusing. A paragraph is missing. I recommend to the authors to read the following articles: - B. Malayappan et al. Quantitative High-Performance Liquid Chromatography–Electrospray Ionization Tandem Mass Spectrometry Analysis of Bis-N₇-Guanine DNA–DNA Cross-Links in White Blood Cells of Cancer Patients Receiving Cyclophosphamide Therapy. Anal. Chem., 2010, 82 (9), pp 3650–3658 -R Mustonen, et al. Measurement by 32P-postlabelling of 7-methylguanine levels in white blood cell DNA of healthy individuals and cancer patients treated with dacarbazine and procarbazine. Human data and method development for 7-alkylguanines. Carcinogenesis (1991) 12 (8):1423-1431. - Whitmire M, et al. LC-MS/ MS Bioanalysis Method Development, Validation, and Sample Analysis: Points to Consider When Conducting Nonclinical and Clinical Studies in Accordance with Current Regulatory Guidances. J Anal Bioanal Tech 2011; S4:001. - European Medicines Agency (2009) EMEA/CHMP/EWP/192217/2009 – Draft Guidance on Validation of Bioanalytical Methods. European	10) Noted and corrected. 11) Noted and corrected. 12) Because both are isomers, to ensure that O₆-Alkylguanine that will be detected, then analysis toward N₇-methylguanine is needed to be conducted for the purpose of selectivity and specificity from the developed method 13) Noted and corrected.
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	Medicines Agency, Committee for Medicinal Products for Human Use. - FDA (2013) Guidance for Industry: Bioanalytical Method Validation. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research. - C Gómez-Canela et al. Liquid chromatography coupled to tandem mass spectrometry and high resolution mass spectrometry as analytical tools to characterize multi-class cytostatic compounds J Chromatography A, 1276 (2013) 78– 94 (there are data for cyclophosphamide) ethical issues- Yes	