



SDI Review Form 1.6

Journal Name:	International Research Journal of Pure and Applied Chemistry
Manuscript Number:	2014_IRJPAC_9391
Title of the Manuscript:	Development and validation of HPLC-UV method for determination of Bovine Serum Albumin and Myoglobin proteins
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:

(<http://www.sciencedomain.org/page.php?id=sdi-general-editorial-policy#Peer-Review-Guideline>)



SDI Review Form 1.6

PART 1: Review Comments

	Reviewer's comment	Author's comment (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)
Compulsory REVISION comments	The following are the comments about the article titled <i>Development and validation of HPLC-UV method for determination of Bovine Serum Albumin and Myoglobin proteins</i>: <i>A reference is needed for the chromatographic conditions used and if it was developed by the authors more discussion is needed about optimization of these conditions e.g optimization the packing material, particle size, flow rate etc.</i> <i>Under robustness results, what about variation in pH does it affect the resolution or the life time of the column.</i> <i>The pH of the mobile phase was never mentioned in the article.</i> <i>It should be mentioned with clear reference on what basis the linearity range was chosen.</i>	- I have developed and optimized the chromatographic conditions of the method which is used for determination of the two proteins. I have added complete and detailed discussion of the optimization procedure, see section 3.1. - As the mobile phase contains trifluoro acetic acid, the apparent pH of the mobile phase for the mixture of acetonitrile and water is acidic (about 2). Actually the mobile phase does not contain a buffer, and the effect of pH was not studied as acidic pH is desired. - I have added the pH of the mobile phase in section 3.1 (method development). - The basis of linearity range of the method for the two proteins is to test the ability of the method for determination/quantitation of the proteins at wide concentration range (from 1 to 1000 ppm). Additionally, the recovery of the two proteins was tested at five concentration levels (1, 100, 300, 500, and 1000 ppm). I have added this in section 3.2.1



SDI Review Form 1.6

	5. <i>A comparison results with at least one of the methods that already exists (reference 6-7) should have been included in the study to complete its applicability.</i> 6. <i>For such a wide range of linearity 1-1000 ppm, more than three concentration levels is required for recovery studies. It is recommended at least five different concentrations. The same applies for repeatability and ruggedness.</i>	(linearity and range). 5. The methods mentioned in the introduction (e.g 6+7) are not used for simultaneous analysis of the two proteins i.e. each method describes determination of one protein. And actually this is the objective of the current work which is to simultaneously analyze the two proteins (in one method). 6. I have added two additional concentrations of the proteins (300 and 500 ppm) in the recovery section (section 3.2.2), repeatability section (see section 3.2.3.1, where I have also added a new table (table 2)), and ruggedness study (see section 3.2.3.2), see the revised manuscript.
<u>Minor</u> REVISION comments		
<u>Optional/General</u> comments		