J. Phar. Res. 2021;20(3):1–4

Print ISSN: 0973-7200
Online ISSN: 2454-8405

Journal of Pharmaceutical Research

RESEARCH ARTICLE

Simultaneous Estimation of Protein and Nucleic Acid Using Derivative Spectrophotometric Method

Atul Agrawal[1], Kiranjot Kaur[1], Rakesh Sharma[1], Amit Goyal[1], Arti Thakkar[1,2,*]

[1] *ISF College of Pharmacy, Ferozepur Road, Ghal Kalan, Moga, 142 001, Punjab, India*
[2] *Amity Institute of Pharmacy, Amity University, Sector: 125, Noida, 201 313, Uttar Pradesh, India*

ARTICLE INFO

Article history:
Received 19.04.2021
Revised 07.08.2021
Accepted 21.08.2021
Published 24.11.2021

* *Corresponding author.*
Arti Thakkar
artirthakkar@gmail.com
arthakkar@amity.edu

https://doi.org/10.54839/v20i3.ms21041

ABSTRACT

Purpose: Simple and precise first derivative zero crossing spectrophotometric method has been developed for the simultaneous estimation of protein and nucleic acid without prior separation. **Approach:** The concentration ranges for protein and nucleic acid were taken in the range of 60-140 μg mL^{-1} and 20-100 μg mL^{-1} respectively in 0.1 M NaOH. Absorption spectra of the samples were recorded between 200 nm to 400 nm against a reagent blank. Zero-order spectra of protein and nucleic acid were stored individually within the above concentration ranges and were derivatized in first order using scaling factor 5 for both the substances. First derivative amplitudes were recorded at 261 nm and 289 nm for estimation of protein and RNA respectively for pure substances and binary mixture. **Finding:** Limit of detection was found to be 2.90 μg mL^{-1} and 0.36 μg mL^{-1} and limit of quantitation was 8.80 μg mL^{-1} and 1.02 μg mL^{-1} respectively for protein and nucleic acid respectively. Precision was found to be 1.38 % for protein and 1.25 % for RNA. Reproducibility was found to be 1.54 % for protein and 1.69 % for RNA. **Conclusions:** Thus, proposed method can be adapted for simultaneous determination of protein and nucleic acid and better alternate technique for immunoassays and electrophoretic methods.

Keywords: First derivative zero crossing spectrophotometry; Protein; Nucleic acid; Ribonucleic acid; Analytical method validation

INTRODUCTION

Nucleic acid and protein analysis holds an important place in the field of life sciences[1] as many downstream changes occurring in each step during genetics, epigenetics, transcriptomics, and proteomics. It is very difficult to perform the qualitative and quantitative measurement of such isolated molecular species, and it is often hindered by the limited availability of sample material. Further, it has been observed that generally nucleic acids and proteins are analysed using Flow Cytometry and Mass Cytometry methods.[2–4] Due to these reasons, there is an increasing interest for simultaneous estimation of nucleic acid and proteins from a single sample.[5] In the present study, we developed a method for the simultaneous estimation of nucleic acid and protein that may be useful for qualitative and quantitative estimation of nucleic acid and protein in a given sample. Through this method the correlation between mRNA expression and its corresponding protein levels can be easily understood. It is a quick and easy procedure for all fields of downstream analysis, especially when using limited sample resources. This method can be very fruitful for estimation of nucleic acid and protein levels in tissues of different ages. It can also be used for over-all screening and functioning of the metabolic machinery of an animal organ by quantitative estimation of nucleic acid and protein.[6,7]

In comparison to chromatographic and electrophoresis methods, spectrophotometric methods are simple, less time consuming and more economic. Under computer-controlled instrumentation, derivative spectrophotometry plays a very important role in the multi-component analysis of mixtures.[8,9] Binary mixtures can be easily resolved by means of a spectrophotometric method, which is based on the simultaneous use of "zero crossing" and "ratio spectra derivative" methods.[10,11] The main aim of the present research work was to investigate the utility of derivative spectrophotometry and to develop reliable spectrophotometric procedure for the simultaneous estimation of protein and

nucleic acid either in laboratory samples, or in dosage forms without prior separation of individual components.

In recent time some of the methods have been published for simultaneous estimation of protein and RNA or DNA. These methods are based on utilizing immunoreactions and DNA hybridizations[12] or using functional nucleic acid molecules[13] or using single molecule arrays.[14] These kinds of methods need specific processing or reagents. The method proposed in the present study is very simple first derivative method for simultaneous estimation of protein and RNA.

MATERIAL AND METHODS

Instruments

Spectrophotometric measurements were made on a Perkin Elmer Lambda 35 UV/VIS Spectrophotometer with UV data processing viewer software version 6.0.3 and UV data processing viewer software version 1.0.0.

Reagents

Bovine Serum Protein and RNA (used as model nucleic acid) were obtained from Jenni Biotech. All these chemicals and solvents were of analytical grade and were used without any further purification. All the required solutions were prepared in double distilled water.

Method

Stock solutions, 1 mg mL^{-1} in 0.1 M NaOH of Standard samples of protein and RNA were freshly prepared individually and further dilutions were made in the same solvent. Binary mixture of protein and RNA (ratio 1:1) was prepared in 0.1 M NaOH. The concentration ranges for protein and nucleic acid were found to be 60-140 μg mL^{-1} and 20-100 μg mL^{-1} respectively. Both analytes were tested for stability in solution and during the actual analysis and the behaviour of the analytes remained unchanged up to about 24 h from their preparation at the room temperature (the spectra was unchanged). Absorbance of the derivative spectra was measured at 261 nm, 289 nm for protein and RNA determination, respectively. Both substances were found to be stable during each kind of experimental measurements. Each measurement was done at room temperature.

First derivative zero crossing spectrophotometry

The absorption spectra of the samples were recorded between 200 nm to 400 nm against a reagent blank (the same of the samples without the substance to be determined) using a 1.0 cm quartz cell. The zero-order spectra of analytes were stored individually within the above concentration ranges and were derivatized in first order using scaling factor 5 for both the substances. The first derivative amplitudes were recorded at 261 nm and 289 nm for estimation of protein and RNA respectively for pure analyte and binary mixture.

Method Validation

The proposed method was validated in the limelight of ICH Guidelines for linearity, range, accuracy, precision, limit of detection and limit of quantification. Consequently, the following were performed.

Linearity and Range

A calibration curve was plotted over a concentration range of 60 to 140 μg mL^{-1} for protein and 20-100 μg mL^{-1} for RNA. The first derivative amplitudes were recorded at 261 nm and 289 nm for estimation of protein and RNA respectively for pure analyte and binary mixture. The Linearity was constructed by plotting concentration against absorbance with each reading.

Accuracy

For studying the accuracy recovery experiments were carried out by the standard addition method. This study was performed by addition of known amounts of protein and RNA to a known concentration of the binary solution. The amounts of standard recovered were calculated in terms of mean recovery with the upper and lower limits of % R.S.D.

Precision

Intra-day precision and inter-day precision for the developed method were measured in terms of % R.S.D. The experiments were repeated three times a day for intra-day precision and on three different days for inter-day precision. Except lowest and highest, five middle concentrations were taken for determination of the precision (concentrations for protein: 70, 80, 90, 100, 120 μg mL^{-1} and RNA: 30, 40, 60, 70, 80 μg mL^{-1}). The concentration values for both intra-day precision and inter-day precision were calculated five times separately and % R.S.D were calculated. Finally, the mean of % R.S.D. (% R.S.D. = [S/X] 100, where S is standard deviation and X is mean of the sample analysed) was reported.

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ were calculated according to the 3s/m and 10s/m criterions, respectively, where s is the standard deviation of the absorbance (n = 10) of the sample and m is the slope of the corresponding calibration curve.

Reproducibility

The reproducibility of the method was determined by the use of different instruments (UV-VIS Spectrophotometer, UV-1700 Pharmaspec, Shimadzu) and different analyst.

RESULTS AND DISCUSSION

The absorption spectra of the two analytes, protein and RNA standards overlapped closely are shown in Figure 1.

For this reason, the determination of these analytes was not possible by direct measurements of absorbance in zero-order spectra. On the other hand, derivative spectroscopy shows more resolution and makes it possible to analyse each analyte without interference of another analyte. Therefore, for simultaneous estimation of both analyte, first derivative zero crossing spectrophotometry method have been developed and validated.

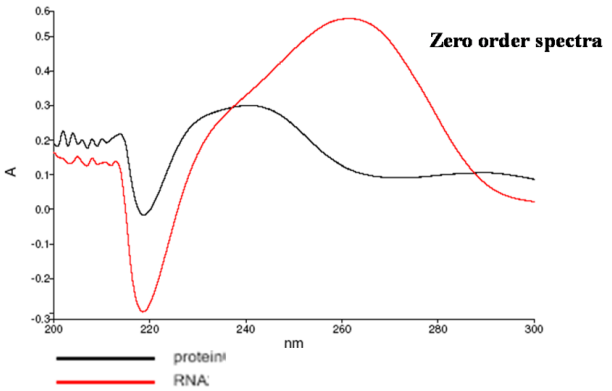

Fig. 1: Zero order spectra ofprotein and RNA Standards.

First derivative zero crossing spectrophotometry

Derivative spectrophotometry has the ability to detect and measure the minor spectral features are enhanced and are measurable. This enhancement of spectra can distinguish very subtle changes in the spectrum and also there is increase in the number of peaks than the zero order spectra that would help in analysing the analyte with more selectively. Thus, it would be helpful to measure analyte easily in the binary mixtures. Thus, in the present method, in contrast to zero-order spectra, first derivative spectra have shown more resolution in terms of zero crossing points shown in Figure 2.

The first derivative wavelengths were 261 nm for protein estimation and 289 nm for RNA estimation. At 261 nm there is no interference of RNA, and protein was determined at 261 nm (Figure 3).

At 289 nm, Protein shows zero absorbance; therefore, RNA was determined without any interference (Figure 4).

The developed method was validated, and all the validation parameters and results of accuracy are shown in Table 1 and Table 2 respectively. The method was found to be linear in the range of 60 – 140 μg mL^{-1} and 20 – 100 μg mL^{-1}. Also, method was precise and reproducible.

CONCLUSION

The developed and validated method provides a simple, fast and reliable means of simultaneous estimation of protein and nucleic acids in formulations containing protein and

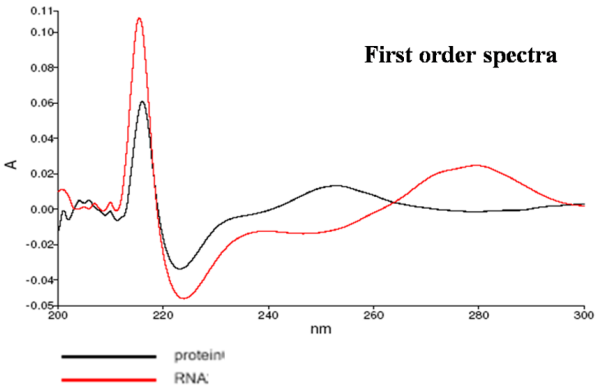

Fig. 2: First derivative spectra of Standardprotein and RNA showing more resolution.

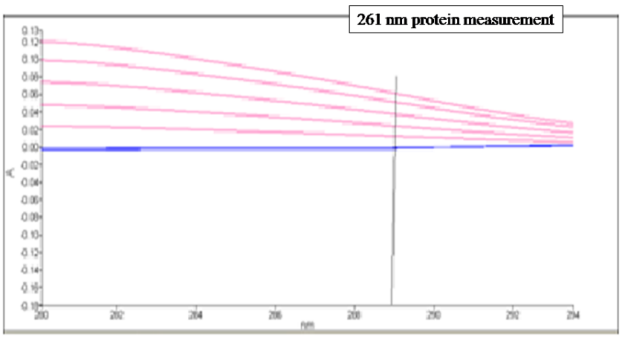

Fig. 3: Protein estimation at 261 nm as there is no contribution of RNA at this wavelength.

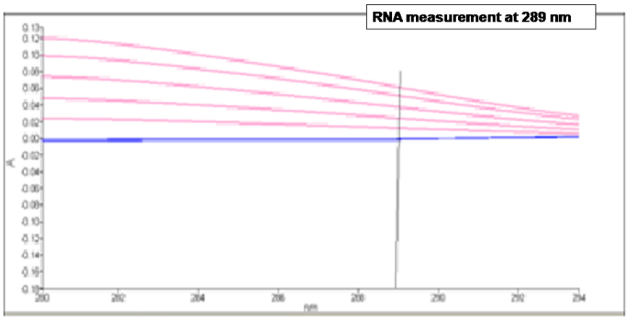

Fig. 4: RNA estimation at 289 nm as there is no contribution of Protein at this wavelength.

Table 1: Validation Parameters

Parameters	Protein	RNA
Range (μg mL^{-1})	60-140	20-100
Slope	0.010	0.062
Intercept	0.029	0.043
Correlation coefficient (R^2)	0.9970	0.9970
Precision (%R.S.D.)	1.38 %	1.25 %
LOD (μg mL^{-1})	2.90	0.36
LOQ (μg mL^{-1})	8.80	1.02
Reproducibility (%R.S.D.)	1.54 %	1.69 %

Table 2: Accuracy Studies

Level of addition (%)	Amount added (μg mL-1)		Amount Found (μg mL-1)		% Recovery	
	Protein	RNA	Protein	RNA	Protein	RNA
80	(48+60) = 108	(32+40) = 72	109.20	70.62	101.12	98.09
100	(60+60) = 120	(40+40) = 80	118.72	77.74	98.93	97.18
120	(72+60) = 132	(48+40) = 88	128.57	87.37	97.40	99.28
% Mean recovery (w/w)					99.15	98.18

nucleic acid without any pre-treatment. This method does not require sophisticated instruments or methods. It is a quick and easy procedure for all fields of downstream analysis, especially when using limited sample resources and ensures reproducible outcome.

ACKNOWLEDGEMENTS

The authors express their sincere thanks to ISF College of Pharmacy, Moga, Punjab (India) for providing the basic research facilities.

REFERENCES

1. Soh KT, Wallace PK. RNA Flow Cytometry Using the Branched DNA Technique. In: Hawley T, Hawley R, editors. Flow Cytometry Protocols. Methods in Molecular Biology. Springer New York. 2018;p. 49–77. Available from: https://doi.org/10.1007/978-1-4939-7346-0_4.
2. Soh KT, Wallace PK. RNA Flow Cytometry Using the Branched DNA Technique. *Methods in Molecular Biology*. 2018;1678:49–77.
3. Lai C, Stepniak D, Sias L, Funatake C. A sensitive flow cytometric method for multi-parametric analysis of microRNA, messenger RNA and protein in single cells. *Methods*. 2018;134-135:136–148. Available from: https://dx.doi.org/10.1016/j.ymeth.2017.12.016.
4. Mavropoulos A, Allo B, He M, Park E, Majonis D, Ornatsky O. Simultaneous Detection of Protein and mRNA in Jurkat and KG-1a Cells by Mass Cytometry. *Cytometry Part A*. 2017;91(12):1200–1208. Available from: https://dx.doi.org/10.1002/cyto.a.23281.
5. Radpour R, Sikora M, Grussenmeyer T, Kohler C, Barekati Z, Holzgreve W, et al. Simultaneous Isolation of DNA, RNA, and Proteins for Genetic, Epigenetic, Transcriptomic, and Proteomic Analysis. *Journal of Proteome Research*. 2009;8(11):5264–5274. Available from: https://dx.doi.org/10.1021/pr900591w.
6. Dean C, Leech RM. Genome Expression during Normal Leaf Development. *Plant Physiology*. 1982;69(4):904–910. Available from: https://dx.doi.org/10.1104/pp.69.4.904.
7. Shibko S, Koivistoinen P, Tratnyek CA, Newhall AR, Friedman L. A method for sequential quantitative separation and determination of protein, RNA, DNA, lipid, and glycogen from a single rat liver homogenate or from a subcellular fraction. *Analytical Biochemistry*. 1967;19(3):514–528. Available from: https://dx.doi.org/10.1016/0003-2697(67)90242-4.
8. Gagandeep, Gill NK, Karan, Sarma GS, Thakkar A. SIMULTANEOUS DETERMINATION OF AMBROXOL, GUAIPHENESIN AND LEVOSALBUTAMOL SULPHATE IN PHARMACEUTICAL FORMULATIONS WITH THE USE OF FOUR RAPID DERIVATIVE SPECTROPHOTOMETRIC METHODS. *Journal of the Chilean Chemical Society*. 2012;57(4):1436–1441. Available from: https://dx.doi.org/10.4067/s0717-97072012000400021.
9. Dave HN, Mashru RC, Thakkar AR. Simultaneous determination of salbutamol sulphate, bromhexine hydrochloride and etofylline in pharmaceutical formulations with the use of four rapid derivative spectrophotometric methods. *Analytica Chimica Acta*. 2007;597(1):113–120. Available from: https://dx.doi.org/10.1016/j.aca.2007.06.035.
10. Gallego JML, Arroyo JP. Spectrophotometric determination of hydrocortisone, nystatin and oxytetracycline in synthetic and pharmaceutical preparations based on various univariate and multivariate methods. *Analytica Chimica Acta*. 2002;460(1):85–97. Available from: https://dx.doi.org/10.1016/s0003-2670(02)00138-1.
11. Nevado JJB, Gallego JML, Peñalvo GC. Spectral ratio derivative spectrophotometric determination of sulphaquinoxaline and pyrimethamine in veterinary formulations. *Journal of Pharmaceutical and Biomedical Analysis*. 1993;11(7):601–607. Available from: https://dx.doi.org/10.1016/0731-7085(93)80011-o.
12. Pagès AM, Safdar S, Ven K, Lammertyn J, Spasic D. DNA-only bioassay for simultaneous detection of proteins and nucleic acids. *Analytical and Bioanalytical Chemistry*. 2021;413(20):4925–4937. Available from: https://dx.doi.org/10.1007/s00216-021-03458-6.
13. Mao X, Gurung A, Xu H, Baloda M, He Y, Liu G. Simultaneous Detection of Nucleic Acid and Protein Using Gold Nanoparticles and Lateral Flow Device. *Analytical Sciences*. 2014;30(6):637–642. Available from: https://dx.doi.org/10.2116/analsci.30.637.
14. Wang X, Walt DR. Simultaneous detection of small molecules, proteins and microRNAs using single molecule arrays. *Chemical Science*. 2020;11(30):7896–7903. Available from: https://dx.doi.org/10.1039/d0sc02552f.