Chapter 6: Summary and Conclusion

The work involved the development and validation of spectrophotometric and chromatographic methods for simultaneous estimation of nitrate and nitrite from complex sample matrices such as water, soil, vegetables and pharmaceutical samples.

6.1 UV-Visible Spectrophotometric Method [1-3]

Following specific conclusions can be drawn from the study of determination of nitrate and nitrite in water and soil samples with composite diazotization-coupling reagents containing Mosapride (MSP) or Metoclopramide (MTCP) as the diazotizable aromatic amines and ethyl acetoacetate (EAA) as the coupling agent.

1. The optimum coupling conditions depend on the nature of the diazo and coupling components used. Spectrophotometric method is simple, inexpensive and reliable but is laborious and time consuming. It provides sufficient skill to the researcher for primary study of nitrate and nitrite estimation.

2. (MSP) or (MTCP) in acid medium react with nitrite to form diazonium ion which is coupled with ethyl acetoacetate (EAA) in basic medium to form azo dye. MSP-EAA is a yellow coloured complex which gives the absorption maxima ($\lambda_{\text{max}}$) at 429 nm. The MTCP-EAA is also yellow coloured complex which gives the absorption maxima ($\lambda_{\text{max}}$) at 437.5 nm.

3. The acidic diazonium salt solution is generally added to the solution of the coupling component. Because, in addition to acid derived from the diazotization process, additional acid is released during coupling, the optimum pH value must be adhered to by adding bases. Alkali metal hydroxide e.g. Sodium hydroxide was used for this purpose. The developed method avoids the use of sodium acetate or formate (weakly acid) or sodium phosphate (weakly alkaline) to buffer the acid. These are added to the reaction mixture before, during, or after combining the components. Because the pH value at the feed point of the diazonium salt solution is always different than that after thorough mixing has been completed, the type of stirrer and speed of stirring are also important in many instances.

4. The sequence in which the two components are combined can also greatly influence the result. The coupling reaction may be completed immediately after the components are mixed or after several hours. If the reaction requires a longer time,
it is advisable to cool with ice and avoid exposure to bright light.

5. To check whether excess diazonium compound is still present, a drop of reaction solution is spotted onto filter paper together with a component that couples readily (e.g., weakly alkaline H acid solution). If no coloration appears, coupling is complete. The presence of unconsumed coupling component can be determined by spotting with a solution of diazonium salt. Attention must also be paid to the volume of coupling solution or suspension. With starting components of low solubility, the physical state is an important factor.

6. The reagents provide a simple and sensitive method for the spectrophotometric determination of nitrite and nitrate for monitoring water pollution.

7. The developed method does not involve any stringent reaction conditions and offers the advantages of color stability about more than 2 hours.

8. The diverse ions added do not interfere in the extraction up to certain concentrations.

9. The statistical analysis of the results by t-tests shows that, there is no significant difference in accuracy and precision between the proposed method and reference method.

10. The precision of the proposed method is evaluated by replicate analysis of samples containing nitrite and nitrate at five different concentrations.

11. The proposed method has been successfully applied to the determination of trace amounts of nitrite and nitrate in soil, water samples, vegetables and pharmaceutical preparations.

12. The proposed method has been successfully applied in Maharashtra Pollution Control Board to determine trace amounts of nitrite and nitrate in soil, water samples, vegetables and pharmaceutical preparations.

6.2 High Performance Liquid Chromatographic Method [4]

Following specific conclusions can be drawn from the RP-HPLC method development for simultaneous estimation of nitrate and nitrite using C-13 column.

1. The results obtained in the validation process and real samples analyses are encouraging and indicate suitability for routine tests. Under established conditions
there are no interferences from sample matrix components. In addition, good separation of constituents under consideration is achieved.

2. The phosphate buffer of pH 3.7 and 20 % methanol as mobile phase is optimum for nitrite and nitrate analysis using RP-HPLC method.

3. The linearity is maintained within a wide range of concentrations. The limit of detection varies from 0.278 and 2.592 ppm thus indicating its significance in low concentration applications such as determination of impurities.

4. The average recoveries of nitrite and nitrate from spiked samples were 98.73 % and 99.03 % respectively, indicating that the method is quite accurate.

5. The developed method is rapid, precise and sensitive and successfully applied for determining nitrite and nitrate amounts in drinking water, common vegetables and pharmaceutical drug samples.

6.3 Ion Chromatographic Method [5]

Following specific conclusions can be drawn from the IC method development for simultaneous estimation of nitrate and nitrite using Dionex IonPac AS18 column.

1. The IonPac AS18 column provides ideal selectivity not only for the separation of nitrite and nitrate, but also for the separation of the analytes from matrix components, which are eluted mostly within mentioned run time.

2. The main advantages of ion chromatography includes the short time needed for analyses, possibility of analysis of small volume samples, high sensitivity and selectivity, and most importantly a possibility of simultaneous separation and determination of nitrate and nitrite ions.

3. This method eliminates the need for time consuming and costly sample pretreatment using reverse phase or ion-exchange cartridges and protein precipitating reagents.

4. Recoveries of nitrite and nitrate were greater than 99 %. Hence this method is quite accurate.

5. The developed analytical method is rapid, simple and sensitive for determining nitrate and nitrite in water, vegetable samples and pharmaceutical preparations.
6.4 Final Conclusion

Among three developed methods, Ion chromatographic method is the best method for the estimation of nitrate and nitrite from water and complex sample matrices like vegetables because it is having the following advantages over spectrophotometric and HPLC methods-

1. It does not require tedious sample pretreatment.
2. It is very rapid method as time of actual sample analysis is few minutes.
3. It allows simultaneous estimation of nitrate and nitrite in presence of interfering ions present in sample matrices.
4. It has highest accuracy among all the three methods studied.
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References:


