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Analytical Development

Development and Validation of an Effective and Alternative Method with UV Spectrophotometer for the Determination of Ammonia Content in Semisolid Dosage Forms Containing D02AE01 Active Substance

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Abstract **Original Research Article**

A new UV-Spectrophotometer method is developed for measurement of Ammonia content using Nessler reagent for semisolid drug products that containing D02AE01 active substance. There are limited published studies evaluating ammonia content for semisolid drug products. The semisolid products have a complex sample matrix which the phases include immiscible or partially miscible liquids and emulsifying agents. The presence of these excipients makes method development more difficult. The aim of our study was to develop and validate a new method for the determination of ammonia content with UV-Spectrophotometer. Meanwhile, an alternative method to the ammonia method available in the British Pharmacopoeia Urea Cream monograph is intended to be promoted. Liquid-liquid phase extraction was used as a preparation technique in semisolid samples. This UV spectrophotometer method is easy applicable and has low cost to perform the analysis. There are meaningful reasons for preferring the UV Spectrophotometer. This instrument gives extremely accurate measurement and it is possible to make measurements quickly, also data analysis is simple. This method was validated for specificity, linearity and range, precision, accuracy and robustness. All results meet the regulatory requirements and this confirmed that the method is suitable for determination of ammonia content for semisolid pharmaceutical dosage forms.

Keywords: Ammonia, Urea, UV-Spectrophotometer, Nessler reagent, Semisolid Drug Products.

1. INTRODUCTION

D02AE01 is an organic compound that is a crystalline form, also known as carbamide. Its formula is H_2NCONH_2. D02AE01increases the moisture-holding properties due to its moisturizing and softening effect, skin with ichthyosis and hyperkeratosis diseases (atopic eczema, xeroderrna, asteatosis and other chronic skin diseases accompanied by skin conditions). Hence, D02AE01contained semisolid drug products are used for medical and therapeutic purposes.[1]

It is also known to use D02AE01 commercially as an NH_3 storage solid compound produced by reacting ammonia with carbon dioxide.[6] D02AE01 generates the weak bases ammonia and carbonate by hydrolysis, thereby consuming acid. [7]

In addition to the positive effects of D02AE01, if the storage conditions of urea contained emulsion and cream products are not observed, the conversion of D02AE01 to ammonia should be considered.

As a result of the hydrolysis of D02AE01, ammonia formation is observed. The active substance of D02AE01 in semisolid drug products turns into ammonia depending on the pH and temperature and may have toxic and corrosive effects for users.[2,3] Therefore, determination of ammonia for containing D02AE01 semisolid drug products is high important.

The methods for D02AE01 mainly are based on photometry dedicated to detection of alkaline products of D02AE01 decomposition using Berthelot or Nessler methods for photometric detection of ammonia formed in the course of enzymatic hydrolysis of D02AE01. [5]

In general, the NH_4^+ concentration of a containing urea emulsions and creams can be determined by a titrimetric method, or a colorimetric method. [4] In

the titrimetric method Ammonia selective electrode is used for the determination of Ammonia. The presence of ammonia in the sample is determined by changing the color with sulfuric acid. Although this method is very practical, it can give misleading results for products in semisolid form. The structure of semisolid drug products consists of highly complex components and ammonia selective electrodes are highly sensitive. Thus, this method may not give reliable results in the long run for the semisolid dosage forms.

In this study, we developed a method by modifying the 204essler method, which is well known for the determination of ammonia with UV Spectrophotometer, for the determination of ammonia in containing D02AE01 active substance semisolid drug products. This method was applied the different real samples with high precision and selectivity.

2. MATERIAL AND METHODS

2.1 Chemicals

Sodium hydroxide, dichloromethane, sodium potassium tartrate tetrahydrate, mercury iodide, potassium iodide and 1M sulfuric acid were purchased from Merck. Sodium sulfate was obtained from Merck.

2.2 Preparation of Standard, Samples and Interfering Chemical Solutions.

2.2.1 Preparation of 1M Sulfuric Acid Solution

1M sulfuric acid solution was made by dissolving 4.2 g sodium hydroxide with 700 ml deionized water in 1000 ml volumetric flask. Then, the solution volume was made up to 1000 ml with deionized water.

2.2.2 Preparation of Rochella Solution

A rochella solution for the experiment was made by dissolving 50 g $KNaC_4H_4O_6.4H_2O$ (Sodium potassium tartrate tetrahydrate in 100 ml deionized water. The part of 30 ml of this solution was boiled and evaporated. Volume of solution was made up to 100 ml.

2.2.3 Preparation of Nessler Reagent

Nessler solution was made by dissolving 100 g mercury iodide and 70 g potassium iodide in 500 ml 0.32 g/ml sodium hydroxide solution and was made up to volume 1000 ml with deionized water. Nessler reagent was filtered throgh from 0.75 µm pre-filter.

2.2.4 Preparation of Diluent and Placebo Solutions

5ml 1M sulfuric acid solution was taken with glass pipette and transferred to centrifuge tube. 10 ml dichloromethan was added to centrifuge tube. This solution was shaked well and centrifuged with 4000 rpm in 5 minutes. Aquoeus phase of this solution was taken and transferred to 50 ml volumetric flask. Then, the solution was made up to 50 ml with deiyonized water. 20 ml of this solution was taken and added 4 ml 1M sodium hydroxide solution and was filtered through 0.45 µm Nylon filter (Milipore Millex-HN) . 10 ml of the filtrate was mixed with 0.2 ml rochella solution and 0.2 ml nessler solution. Plasebo solution was prepared in the same manner but including a quantity of placebo.

2.2.5 Preparation of Stock Standard Solution

A stock standard solution was obtained by dissolving 175 mg ammonium sulfate in 100 ml deionized water. Ammonium sulfate concentration of stock solution was 1.75 mg/ml.

2.2.6 Preparation of Standard Solution

The working standard solution was made by diluting the stock standard solution to 0.175 mg/ml ammonium sulfate solution concentration. 5 ml of standard solution was taken with glass pipette and transferred to centrifuge tube. 10 ml dichloromethane was added to centrifuge tube. This solution was shaked in 20 minutes with mechanical shaker. At the and of the shaking step, the solution was centrifuged with 4000 rpm in 5 minutes. 3ml of the aqueous phase of the solution was taken and transferred to 50 ml volumetric flask. Then, the solution was made up to 50 ml with deiyonized water. 20 ml of this solution was taken and added 4 ml 1M sodium hydroxide solution and was filtered through 0.45 µm Nylon filter (Milipore Millex-HN) . 10 ml of the filtrate was mixed with 0.2 ml rochella solution and 0.2 ml nessler solution.

2.2.7 Preparation of Sample Solution

Each of semisolid drug samples (0.2 g) were mixed with 5 ml 1M sulfuric acid solution and 10 ml dichloromethan in centrifuge tube. This solution was shaked in 20 minutes with mechanical shaker. At the and of the shaking step, the solution was centrifuged with 4000 rpm in 5 minutes. 3ml of the aqueous phase of the solution was taken and transferred to 50 ml volumetric flask. Than, the solution was made up to 50 ml with deiyonized water. 20 ml of this solution was taken and added 4 ml 1M sodium hydroxide solution and was filtered through 0.45 µm Nylon filter (Milipore Millex-HN) . 10 ml of the filtrate was mixed with 0.2 ml rochella solution and 0.2 ml nessler solution.

2.3 Experimentation and Method Conditions

A UV-Visible Spectrophotometer, the brand of Varian, was used in this study. The determination wavelength of ammonia was 405 nm with the UV spectrophotometer. The measurement of cell size was 10 mm.

3. RESULTS AND DISCUSSION

3.1 Method development

A rapid, simple and specific method using UV Spectrophotometer was developed and validated by using specificity, linearity, precision, accuracy and robustness parameters according to the International Conference on Harmonization (ICH) requirements.

A specificity test was performed to detect the ability of the method to measure only the substances that

were aimed to measure in the analyzed samples. Diluent, placebo, standard, samples and spiked samples were used in this study. There should be no interference from diluent and placebo solutions. The linearity of the method was evaluated by analyzing two replicates for each of the six calibration standards. The linearity range was between 0.002 mg/ml and 0.361 mg/ml ammonium sulfate concentrations. The repeatability was determined by analyzing two replicate finished product samples and six replicates of spiked finished product samples. The intermediate precision was performed with analyzing two replicate finished product samples and six replicates of spiked finished product on a different day from repeatability study.

Accuracy of method was demonstrated by adding ammonium sulfate into the samples at the levels of LOQ, 100% and 120%. Total 9 samples, 3 samples for each level, were prepared. Accuracy of each sample studied should be in the range of 95.0% -105.0% and should not exceed ±5% deviation for finished product samples.

Ammonium sulfate standard and semisolid product solutions were freshly prepared, and they were analyzed at certain time intervals keeping storing conditions constant. Recording the absorbances obtained, % conformity was calculated.

3.2 Method validation

3.2.1 Limit of detection (LOD) and limit of quantification (LOQ)

UV absorbances of solutions prepared at 6 different concentrations between 1% and 120% of specification were measured. Ammonia was found to be linear between 0.0037 mg/ml and 0.3663 mg/ml. Correlation coefficient (R) was found 1.0000 for Ammonia. Correlation coefficient is more than 0.99. Limit of detection (LOD) value of ammonia was found 0.0012 mg/ml and Limit of Quantification (LOQ) value was found 0.0037 mg/ml.

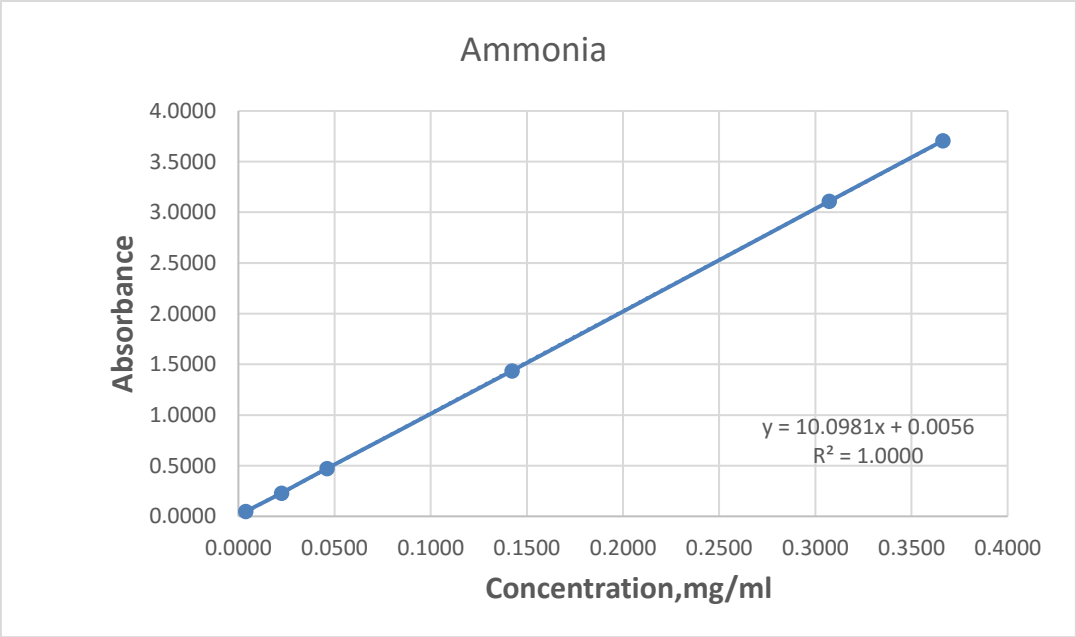

Figure 1. Linearity graphic of ammonia

Table 1. The linearity, detection limit, and quantitation limit of Ammonia

Compound	Concentration (mg/ml)	Slope	Intercept	Correlation Coefficient (R)	Limit of Detection (mg/ml)	Limit of Quantification (mg/ml)
Ammonia	0.0024 - 0.0224-0.0461-0.1423-0.3072-0.3663	10.10005 (± 0.0369)	0.005071 (± 0.076)	1.0000	0.0012	0.0037

3.2.2 Precision

3.2.2.1 System Precision

Performed six consecutive absorbance measurement of standard solution prepared at 100 % concentration. Recorded the peak absorbances and relative standard deviation (RSD %) between the standards obtained from measurements. Observed RSD% value is 0.06 from six consecutive absorbance measurement.

Table 2: The system precision results

Measurement No	Absorbance
1	0.5012
2	0.5012
3	0.5019
4	0.5013
5	05011
6	0.5016
MEAN	**0.5014**
SD	**0.0003**
% RSD	**0.06**
CONFIDENCE INTERVAL (95%)	**0.5014 ± 0.0002**

3.2.2.2 Repeatability and Intermediate Precision

To prove precision of the method six spiked samples are prepared and analysis were performed. Relative standard deviation (RSD%) for obtained from 6 samples were found as 0.87 and 1.13 for ammonia in the emulsion and cream spiked samples respectively. Repeatability and intermediate precision of the method was proved. All results including individual data, SD and confidence interval are in the table 3.

Table 3. Repeatability and intermediate precision results and RSD (%) values of semisolid drug samples

Dosage Form	Compound	Mean Result of Repeatability (Ammonia %) (n=6)	RSD (%)	Mean Result of Intermediate Precision (Ammonia %) (n=6)	RSD (%)
Semisolid (emulsion form)	Ammonia	0.187	0.87	0.198	0.59
Semisolid (cream form)		0.191	1.13	0.192	1.79

3.2.3 Accuracy

Accuracy of method was demonstrated adding ammonium sulfate into semisolid sample solutions. Totally 9 samples (3 samples for each level; LOQ, 100% and 120%) was prepared and measured by UV spectra twice. Recovery of each sample is in the range of 90.0% and 110.0%. Relative standard deviation (RSD%) obtained from 9 samples were found as 2.24 and 1.46 for semisolid form-1 and semisolid form-2 respectively. RSD (%) values nine samples of semisolid drug samples were found lower than 4.0 %. All individual results including RSD% values are in the table 4.

Table 4: Accuracy results of cream and emulsions

Dosage Form	Compound	Theoretical Concentration (%)	Mean Result (Ammonia %)	RSD (%)
Semisolid (emulsion form)	Ammonia	LOQ	101.5	2.24
		100	97.8	
		120	96.7	
Semisolid (cream form)		LOQ	103.7	1.46
		100	101.4	
		120	102.4	

3.2.4 Robustness

In order to demonstrate the Robustness of ammonia content method, the effect of changes in the method parameters and solution stability were examined. For this aim parameters which listed below were performed:

Change in method parameter

Changed parameter which listed below were performed:
Wavelength : 403 nm and 407 nm
Results showed that there was no significant change in ammonia content results for semisolid drug products in Table 5. The method is robust in view of changes to wavelength.

Table 5: Change in method parameter results of Semisolid form-1 and Semisolid form-2 samples – Robustness parameters

Parameters	Ammonia content for semisolid form-1 (%)	Ammonia content for semisolid form-2 (%)
Repeatability result	0.192	0.136
Wavelength 403 nm	0.182	0.132
Wavelength 407 nm	0.179	0.147

Solution stability

Spiked sample and standard solutions were prepared and analyzed at certain time intervals keeping

store conditions constant and percentage of conformity was calculated. Percentage of conformity were not in the range of 95.0% and 105.0% after one hour at 25 ºC for standard and sample solution respectively. Standard and sample solutions were not stable at these conditions. Standard and sample solutions should prepare freshly. Solution stability results are in the Table 6

Table 6. Solution stability results of semisolid form-1 and semisolid form-2 samples – Robustness parameters

Dosage Form	Percentage of Conformity (%)	Absorbance (Initial)	Absorbance (1 Hour)
Semisolid (emulsion form)	94.7	0.5506	0.5217
Semisolid (cream form)	88.9	0.6264	0.5571

4. CONCLUSIONS

The proposed UV spectrophotometer method successfully applied for studying ammonia content determination in semisolid drug products with D02AE01 active ingredient. This method revealed the ammonia content in semisolid dosage forms which have a complex sample matrix. Ammonia content was determined with liquid-liquid phase extraction method by passing the active substance from the oil phase to the water phase. Using of the extraction method increases the sensitivity and the selectivity of the UV-Vis methodology. [8] Then, Nessler reaction is applied for the obtained water phase. The Nessler reaction reveals ammonia if there is a presence of ammonia in the sample solution. Using Nessler reaction method for detecting the ammonia content removes the need for an additional ammonia content detecting device. The validation of proposed method was done in accordance with ICH guidelines and proved that the method to be simple, precise, reliable, and robust. Carrying out the ammonia content method by UV spectrophotometer has benefited from its easy applicability and being a more accessible instrument.

REFERENCES

1. T.Doi, K. Kajimura, S. Takatori, N. Fukui, S. Taguchi, S. Iwagami. Simultaneous measurement of diazolidinyl urea, urea, and allantoin in cosmetic samples by hydrophilic interaction chromatography. Journal of Chromatography B
2. F. Lu, G. G. Botte. Understanding the Electrochemically Induced Conversion of Urea to Ammonia Using Nickel Based Catalysts. Electrochimica Acta
3. K. Lin, Y. Zhu, Y. Zhang, H. Lin. Determination of ammonia nitrogen in natural waters: Recent advances and applications. Trends in Environmental Analytical Chemistry
4. H. Jeong, J. Park, H. Kim. Determination of $NH4^+$ in Environmental Water with Interfering Substances Using the Modified Nessler Method. Hindawi Publishing Corporation, Journal of Chemistry, 2013, , 9 pages
5. J. Bzura, R. Koncki. A Mechanized Urease Activity Assay. Enzyme and Microbial Technology, 2019, pages (1-7)
6. G. Prieto, Y. Litsuka, H. Yamauchi, A. Mizuno, O. Prieto, C. R. Gay. Urea in Water-in-Oil Emulsions for Ammonia Production. Department of Ecological Engineering, Toyohashi University of Technology, Japan Department of Chemical Engineering, National University of Tucumán, Argentine Republic
7. M. Djerdjev, P. Priyananda, J. Gore, J. K. Beattie, G. Neto. Safer emulsion explosives resulting from NOx inhibition. Chemical Engineering Journal, 2021, 403
8. M. L. C. Passos, M. Lucia M.F.S Saraiwa, Detection in UV-visible spectrophotometry: Detectors, detection systems, and detection strategies, Measurement, Volume 135, March 2019, Pages 896-904