Spectrophotometric Determination Of Dimethylamine In Metformin Hydrochloride Via Derivatization With 1-Flouro 2,4-Dinitrobenzene.

Ramakant Sharma[1*], Manmeet Singh Saluja[2]

[1*]Research Scholars, Department of Pharmacy, Monad University, Hapur, U.P.
[2]Professor, Department of Pharmacy, Monad University, Hapur, U.P.

***Corresponding author:** Ramakant Sharma
[*]Research Scholars, Department of Pharmacy, Monad University, Hapur, U.P.

Abstract:
This abstract introduces an analytical methodology that has been developed to conduct a thorough study of pharmaceutical items, beyond the limitations of single-point measurements, in order to evaluate their quality. Our analytical methodology integrates many advanced methods to provide a comprehensive evaluation of pharmaceutical samples, enabling the identification and measurement of active components and contaminants across different concentration ranges. This methodology not only detects the existence of the active component but also investigates the intricate details of its chemical composition and level of purity.

1. Introduction:

This introduction provides an overview of the importance of using dependable techniques for the analysis of pharmaceutical goods, with a particular focus on the detection and characterization of active components and contaminants. The aim of this study is to elucidate the essential role that these procedures play in guaranteeing the overall quality and safety of pharmaceutical products. Aim of this research work was to develop and validate reliable analytical methods for determining the presence ofactive ingredients and impurities in pharmaceutical dosage forms and bulk medicines. the oral antidiabetic medication metformin (N, N-dimethyl imidodicarbon imidicdiamide). For those who are overweight or obese and have normal renal function, this medication is the first line of defense against type 2 diabetes [1-3]. Metformin may help reduce diabetes-related cardiovascular and perhaps cancer problems, according to preliminary research [4-6]. Dimethyl amine has one proton and two methyl groups attached to a nitrogen atom. The ammonium salt of dimethylamine has a pKa of 10.73, making it a weak base, whereas the pKa of trimethylamine is 9.79.Acids react with dimethyl amine to produce salts like dimethylamine.

2. Materials and method:

Instrument

According to the reviewed literature, numerous techniques were available for the detection of dimethyl amine concentration, including ionic chromatography (IC), gas chromatography (GC), and capillary gas chromatography-mass spectrometry. The current objective is to quantify derivitized Metformin HCl drug substance and drug product dimethyl amine levels using UV spectrophotometry. UV Spectro photometry is a common technique of examination because to its ease of use, low cost, and short analysis time.

Chemicals and reagents

Metformin HCl was provided by our APL Research Centre-II. Dimethyl amine. HCl analytical grade procure from Merck Chemicals, India. 1-Fluro-2, 4 dinitro benzene procure from sigma Aldrich. Triethyl amine analytical grade procure from Spectrochem. Acetonitrile analytical grade procure from Merck Chemicals, India.

Methods

Method development

Dimethyl amine is a common reagent used in the synthesis of several pharmaceuticals. Various analytical methods have allowed for its detection and quantification. The chemical compound dimethyl amine reacts with acids and oxidizing agents. When fluorodinitrobenzene reagent solution is mixed with dimethyl amine, no color develops. When diethyl amine was added to the solution, a color developed in the presence of the weak base, but no absorbance spectrum was produced.

Triethyl amine is a weak base that forms quaternary salts of colors when combined with amines. Triethylamine and fluorodinitrobenzene reagent solutions, each 100 l, were thoroughly combined. One milliliter of a 0.15 mg/ml dimethylamine solution was added, followed by three milliliters of acetonitrile and a final volume of ten milliliters. Maximal absorbance at 378 nm was found after scanning the solution from 300 to 500 nm in the absence of a blank.

Preparation of solutions
Creating a reagent solution using fluorodinitrobenzene In 100ml of acetonitrile, I dissolved 100mg of 1-fluoro-2, 4-dinitrobenzene.

Standard solution
In acetonitrile, we have dissolved 0.15 g of dimethyl amine. To a 10 ml volumetric flask, I poured 100 l of triethylamine solution, 100 l of fluorodinitro benzene reagent solution, 1 ml of 0.15 g/ml dimethylamine solution, 3 ml of acetonitrile, and stirred the mixture until it reached 10 ml. Causes chromogen to be produced that gives off a yellow hue. We used 0.45-micron filter paper to refine the solution.

Sample solution
Transferred 100µlof each triethylamine solution and fluorodinitro benzene reagent solution into a 10ml volumetric flask swirled well. Accurately weighed and transferred 0.1g of Metformin HCl, added 3ml acetonitrile mixed well made up to 10ml with acetonitrile. Filtered the solution with 0.45µ filter paper.

Evaluation of blank solution
To a 10-milliliter volumetric flask, add 3 milliliters of acetonitrile to a well-stirred solution of triethylamine and the reagent solutions (100 milliliters total).
Filtered the solution with 0.45µ filtered paper.

Procedure
We scanned the standard and sample solutions for their yellow chromogen between 300 and 500 nm, accounting for blank readings. Maximum absorbance at 378 nm was observed for the dimethyl amine derivative.Maximum areas of absorbency, as seen in Figure 2.5.

$$\text{Dimethyl amine (\%w/w)} = \frac{AT}{As} \times \frac{Ds}{DT} \times P \times F$$

Where
AT is absorbance of sample solution,
AS is the absorbance of standard solution,
DS is the dilution factor of standard solution (Weight÷Dilution),
DT is the dilution factor of Sample solution (Weight÷Dilution),
P is the potency of the Dimethyl amine. HCl
F is the (Mwt of Dimethyl amine ÷ Mwt of Dimethyl amine. HCl)

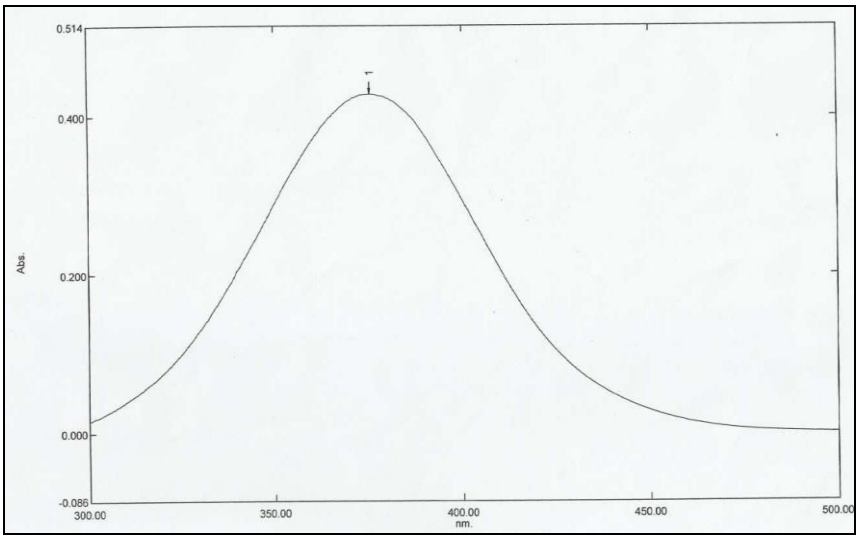

Figure 1 UV Spectrum of derivatized dimethylamine

Spectral characteristics:
Absorbance at 378 nm was measured against the concentration of a standard solution of dimethylamine coupled with fluorodinitro benzene in the presence of a weak base (triethylamine). Good agreement with Beer's law is shown between absorbance and dimethylamine concentration across the concentration range of 8 g/g to 230 g/g.

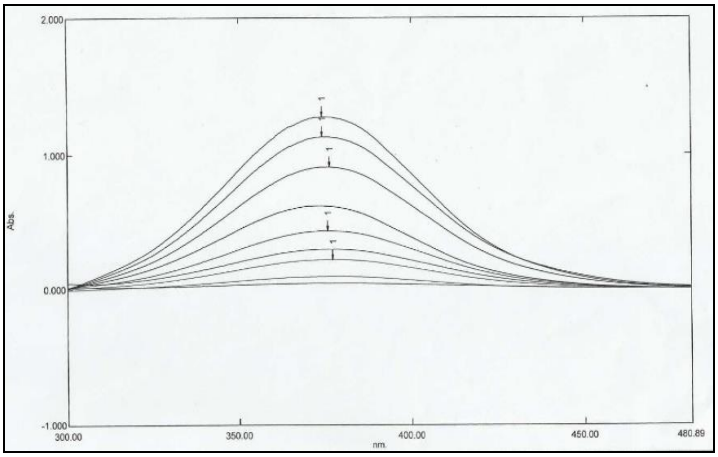

Figure 2 Overlay spectrum of derivatized dimethylamine at different concentrations**.**

3. Results and discussion

Method development

The concentration of the dimethylamine derivative causes a linear increase in solution color. Dimethylamine levels may be calculated from here. We detected very little concentrations of dimethylamine in the medicinal material. The color reagent was made using the trial-and-error approach. Acetonitrile containing 5mg/ml of the fluorodinitrobenzene reagent was produced. Fluorodinitrobenzene and triethyl amine were added to 100 ml aliquots of the normal dimethyl amine solution. The steady yellow hue appeared almost instantly at room temperature. The highest absorption of the generated yellow color occurs at a wavelength of 378 nm. The transmittance drops as the concentration of the reagent solution or triethyl amine rises.

Method Validation

The analytical method's performance characteristics are verified in accordance with ICH recommendations [10] by a series of laboratory investigations known as validation. Linearity, accuracy, system precision, intraday precision, detection limit, and quantification range were all verified for the procedure.

Precision

System precision

Dimethylamine absorbance at 378 nm was recorded six times with a relative standard deviation (RSD) of less than one, indicating satisfactory repeatability and, by extension, accuracy of the system. The accuracy metrics for the system are shown in Table 1.

Table 1 System precision results of dimethylamine

n	Absorbance
1	0.89
2	0.895
3	0.892
4	0.898
5	0.895
6	0.89
Mean	0.893
SD^	0.003
% RSD*	0.358

^ Standard deviation,* Relative standard deviation

Method precision

Metformin HCl drug substance was spiked with a known concentration of dimethylamine, and the method's repeatability and intermediate precision were evaluated over the course of three consecutive days using three separate analysts at the same working concentration.

Repeatability (Intra day precision)

The method developed is method precise by the test of repeatability, as shown by the % RSD of less than 1 for six consecutive recordings of absorbance at 378 nm of the dimethylamine from the same homogeneous mixture at working concentration (Table 2).

Table 2 Intraday precision results of dimethylamine (154 µg/g) spiked in Metformin HCl drug substance

n	**Dimethylamine content (µg/g)**
1	154.2
2	154.5
3	154.8
4	155.1
5	153.9
6	155.6
Mean	154.7
SD^	0.618
% RSD*	0.399

^ Standard deviation,* Relative standard deviation

Intermediate Precision (Inter day precision / Ruggedness)

The method developed is inter day precise / rugged (Table 3) as shown by the % RSD being less than '1' when recording absorbance at 378 nm of the dimethylamine from the same homogeneous mixture at working concentration on three consecutive days by three different analysts.

Table 3 Inter day precision results of dimethylamine (154 µg/g) spiked in Metformin HCl drug substance.

n	Dimethylamine Content (µg/g)		
	Day 1	Day 2	Day 3
1	154.6	153.9	154.2
2	154.9	153.7	153.9
3	154.2	154.2	154.6
4	154.7	153.9	154.1
5	154.9	154	154
6	154.4	153.9	154.3
Mean	154.6	153.9	154.2
SD^	0.279	0.163	0.248
% RSD*	0.18	0.106	0.161

^ Standard deviation,* Relative standard deviation

Linearity:

Derivatized dimethylamine standard solutions ranging in concentration from 8 g/g to 230 g/g were made. The absorbance at 378 nm was correlated with the dimethylamine concentration to create a calibration curve. As can be seen in (Table 4), there is a strong relationship between absorbance and dimethylamine concentration across a wide concentration range. The approach is considered linear from 8 g/g to 230 g/g since the correlation coefficients were larger than 0.999, which is the acceptance criterion for method validation.

Table 4 Calibration data for dimethylamine standard

N	Dimethylamine Standard (µg/g)	Absorbance
1	8	0.045
2	15	0.091
3	46	0.293
4	77	0.426
5	154	0.898
6	184	1.12
7	230	1.295
Regression equation		y = 0.005x + 0.012
Correlation coefficient (r2) 0.999		

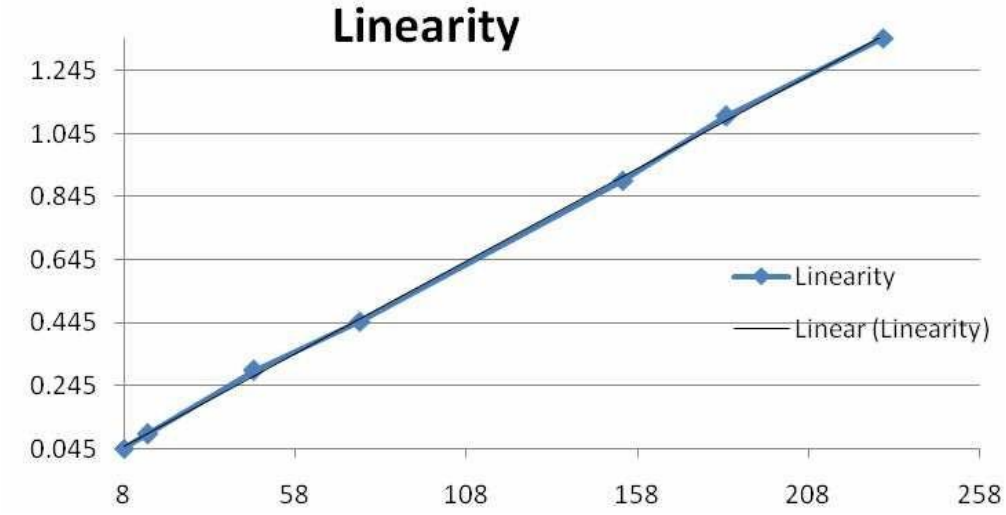

Figure 7 Linearity graph of Dimethyl amine content.

Accuracy

Recovery studies were used to assess accuracy by calculating the mean recovery percentage across three distinct sample concentrations (LOQ150%). Three separate checks were done on each stage. According to (Table 5), we can determine the median percentage of recovery. All observed data fall within the minimum and maximum ranges for acceptable recovery, proving the reliability of the established approach.

Table 5 Recovery results from spiking of Metformin HCl drug substance with dimethyl amine

Accuracy (Average of Triplicates)	Level-I	Level-II	Level-III
	(LOQ)	-100%	-150%
Added (µg/g)	46.65	155.5	233.3
Found (µg/g)	46.48	152.7	230.9
Recovery (µg/g)	99.64	98.05	98.99
% RSD	0.76	0.26	0.3

Robustness

The robustness of an analytical technique is its ability to withstand routine use, which may include tiny but intentional changes to the method's parameters. Despite introducing intentional alterations into the mix of the colored reagent and solvents, it was observed that the % RSD for the dimethylamine concentration was less than 2, indicating that the approach was robust.

Sensitivity

Limits of quantitation (LOQ) and limits of detection (LOD) were calculated to approximate the sensitivity of the proposed approach for measuring dimethylamine concentration. It was determined that 15 ng/g was the lower limit of detection (LOD) and 46 ng/g was the lower limit of quantitation (LOQ). Table 6 summarizes the findings of the optical characteristics study.

Table 6 Optical characteristics of dimethylamine content in Metformin HCl drug substance

S.No	Parameters	Dimethyl amine
1	Wavelength measurement	378nm
2	Beer's law limit (µg/g)	8-230
3	Regression equation (y=mX+C)	Y=0.005x+0.012
4	Slope	0.005
5	Intercept	0.012
6	Correlation Coefficient (R^2)	0.999
7	Precision(%RSD)	
	Intraday (n=6)	0.149
	Interday (n=6)	0.399
8	Accuracy (%Recovery)(For API)	98.9
9	Accuracy (%Recovery)(For Formulation)	98.1
10	LOD(µg/g)	46
11	LOQ(µg/g)	15

4. Conclusion

With the help of ICH recommendations, a quick UV spectrophotometric technique for the quantitative determination of dimethylamine in Metformin HCl drug substance was developed and validated, resulting in reduced testing costs. Dimethylamine showed linearity in the range of 8 g/g to 230 g/g when the described technique was used. Relative standard deviations of 0.149% and 0.399%, respectively, demonstrate the accuracy both between and within days. In experiments measuring precision, the average percentage of data recovered was found to be between 98% and 101%. Both 15 ng/g and 46 ng/g were determined to be the limits of detection (LOD) and quantitation (LOQ) respectively. We conclude that the devised UV spectrophotometric technique for routinely analyzing dimethylamine concentration in Metformin HCl medicinal substances is accurate, precise, linear, rugged, and durable.

5. References

1. International Conference on Harmonization (2000) Draft Revised Guidance On Impurities In New Drug Substances. Federal Register Q3A(R) 65 (140): 45085.
2. International Conference on Harmonization (2000) Draft Revised Guidance On Impurities In New Drug Products. Federal Register Q3B(R) 65 (139) 44791.
3. International Conference on Harmonization (1997) Impurities, Q3C Guidelines for Residual Solvents, Q3C. Federal Register 62 (247) 67377.
4. International Conference on Harmonization (1999) Specifications, Q6A: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products. Chemical substances 65 (146) 67488.
5. S. Ahuja, Impurities Evaluation of Pharmaceuticals. Marcel Dekker, New York, (1998), p. 142.
6. T.N. Riley, Steric aspects of drug action. Pharmacist 23 (1998) 40.
7. Harry W. Lewis and Christopher J. Moody (13 Jun 1989). Experimental Organic Chemistry: Principles and Practice (Illustrated ed.). Wiley Blackwell. pp. 159–173. ISBN 978-0-632-02017-1.