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Spectrophotometric method for determination of dimethylamine in metformin hydrochloride via derivatization with 1-fluoro 2,4-dinitrobenzene

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ABSTRACT

A rapid, simple and sensitive UV Spectrophotometric method has been developed for the quantitative estimation of dimethylamine in Metformin HCl drug substance and drug product .and validated as per ICH guidelines. The method was based upon the observation, that a characteristic colour results via dervatization with 1-Fluro2,4-dinitrobenzene. The addition of Flurodinitrobenzene reagent in dimethylamine dissolved in acetonitrile in the presence of triethyl amine results in the formation of yellow colour showing absorbance maxima at λ378nm. The developed method resulted as dimethylamine exhibiting linearity range 8 to 230 µg/g. The interday and intraday precision is exemplified by relative standard deviation of 0.149 % and 0.399%. .Percentage of mean recovery was found in the range of 98-101% during accuracy studies. The LOD and LOQ were found to be 15µg/g and 46µg/g respectively.

Keywords: UV Spectrophotometry, Dimethylamine HCl, Metformin HCl, Method development, Validation

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INTRODUCTION

Metformin (N,N-dimethylimidodicarbonimidic diamide)(Fig.1) is an oral antidiabetic drug in thebiguanide class. It is the first-line drug of choice for the treatment of type 2 diabetes, in particular, in overweight and obese people and those with normal kidney function.[1-3] Limited evidence suggests metformin may prevent the cardiovascular and possibly the cancer complications of diabetes.[4-6]

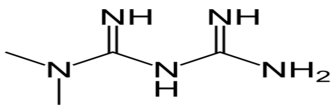

Fig. 1: Chemical Structure of metformin

The usual synthesis of metformin, originally described in 1922 and reproduced in multiple later patents and publications, involves the reaction of dimethylamine hydrochloride and2-cyanoguanidine (dicyandiamide) with heating.[7,8] (Fig.2). Metformin drug preparations must be tested for residual dimethylamine, because the weekly acidic conditions required for the reaction can promote formation of dimethyl nitraso amine, suspected to carcinogen[9] . Dimethylamine undergoes nitrosation under weak acid conditions to give dimethylnitrosamine. This animal carcinogen has been detected and quantified in human urine samples and it may also arise from nitrosation of dimethylamine by nitrogen oxides present in acid rain in highly industrialized countries.

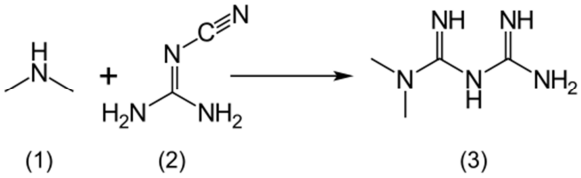

Fig. 2: Dimethylamine used in the synthesis of metformin

MATERIALS AND METHODS

Instrument

A double beam UV-visible spectrophotometer (Shimadzu, model 1800) having two matched quartz cells with 1 cm light path and loaded with UV probe software (version 2.41) was used for recording of spectra and measuring absorbance.

Chemicals and reagents:

Pure drug Metformin HCl was provided by our APL Research Centre-II . (A Division of Aurobindo Pharma Ltd). All the reagents and chemicals used were of analytical grade from Merck Chemicals, India. 1-Fluro-2,4 dinitro benzene reagent from sigma aldrich.

Methods:

Preparation of solutions:

Standard solution:

Dissolve 0.15µg/ml of dimethylamine in acetonitrile. Transfer 100µl of each triethylamine solution and reagent solutions swirl well, added 1ml of 0.15µg/ml dimethylamine solution add 3ml of acetonitrile mix well made up to 10ml with acetonitrile. Results in the formation of yellow colour chromogen. Filter the solution with 0.45µ filter paper.

Sample solution:

Transfer 100µlof each triethylamine solution and reagent solutions swirl well. Accurately weigh and transfer 0.1g of Metformin HCl,add 3ml acetonitrile mix well made up to 10ml with acetonitrile. Filter the solution with 0.45µ filter paper.

Blank:

Transfer 100µlof each triethylamine solution and reagent solutions swirl well, add 3ml acetonitrile mix well made up to 10ml with acetonitrile. Filter the solution with 0.45µ filter paper.

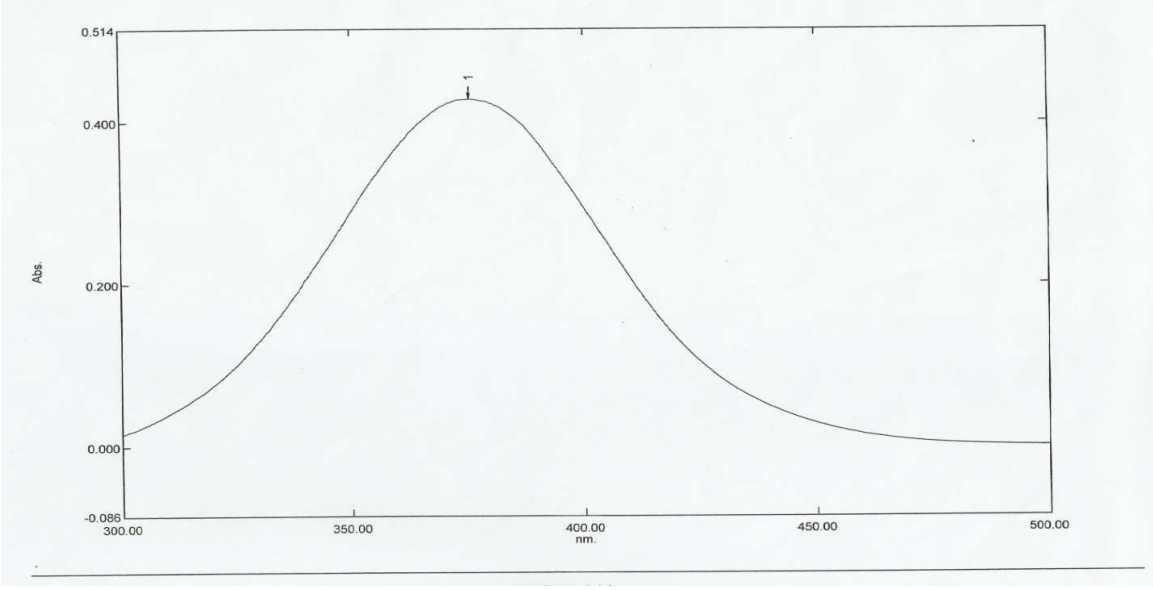

Fig. 3: UV Spectrum of derivatized dimethylamine

Procedure:
Scanned the yellow colour chromogen of standard and sample solution between 300 nm to 500nm with blank correction the absorbance maxima 378nm. Fig.3

Spectral characteristics:
Prepared standard solution of dimethylamine coupled with fluorodinitro benzene in the presence of week base (triethylamine) at different concentration versus corresponding absorbance at 378nm. The results showing an excellent correlation between absorbance and concentration levels of dimethylamine within the concentration range of 8µg/g to 230µg/g show good agreement with Beer's law. Fig. 4.

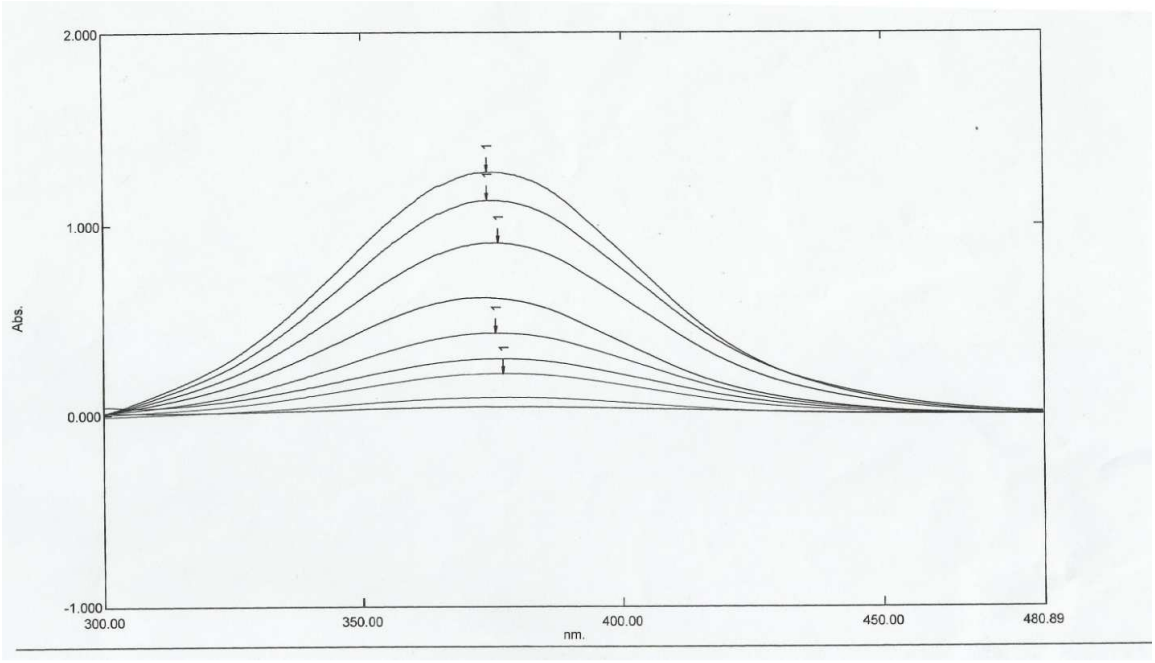

Fig. 4: Overlay spectrum of derivatized dimethylamine at different concentrations

RESULTS AND DISCUSSION

Method development:
The intensity of the color of the solution is proportional to the dimethylamine derivative concentration. This is the basis to determine the content of dimethylamine. We measured the dimethylamine content in drug substance low levels. On the basis of trail and error method colour reagent is prepared. The 5mg/ml of fluorodinitrobenzene reagent had prepared in acetonitrile. Transfer 100µl each of flurodinitrobenzene, triethyl amine to the aliquots of the standardized dimethyl amine solution. At a room temperature yellow color developed immediately which is stable . The developed yellow colour has its absorption maximum at λ 378nm. If increasing the concentration of reagent solution or triethyl amine, decreases the transmittance.

Method Validation:
Validation of the analytical method is the process that establishes by laboratory studies in which the performance characteristics of the method was validated as per ICH guidelines.[10] The method was validated for the parameters Linearity, Accuracy, System precision, intraday precision, Limit of Detection, Limit of Quantification.

Precision
System precision
Six replicate recording of absorbance at 378 nm of standard solution at working concentration showed % RSD (Relative Standard Deviation) less than 1 concerning absorbance for the dimethylamine, which indicates the acceptable reproducibility and thereby the precision of the system. System precision results are tabulated in (Table 1).

Table 1: System precision results of dimethylamine

n	Absorbance
1	0.890
2	0.895
3	0.892
4	0.898
5	0.895
6	0.890
Mean	0.893
SD^	0.003
%RSD*	0.358

^ *Standard deviation*
* *Relative standard deviation*

Method precision

Method precision was determined by performing content of dimethylamine by spiking with known concentration of dimethylamine in Metformin HCl drug substance under the tests of (i) repeatability (Intra day precision) and (ii) Intermediate precision (Inter day precision) performed during 3 consecutive days by three different analysts, at working concentration.

Repeatability (Intra day precision)

Six consecutive recording of absorbance at 378 nm of the dimethylamine from the same homogeneous mixture at working concentration showed % RSD less than 1, which indicate that the method developed is method precise by the test of repeatability and hence can be understood that the method gives consistently reproducible results (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

Intermediate Precision (Inter day precision / Ruggedness)

Six consecutive recording of absorbance at 378 nm of the dimethylamine from the same homogeneous mixture at working concentration on three consecutive days by three different analysts, showed % RSD less than 1 within and between days, which indicate the method developed is inter day precise / rugged (Table 3).

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

n	Day 1	Dimethylamine Content(µg/g) 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	154.0	154.0
6	154.4	153.9	154.3
Mean	154.6	153.9	154.2
SD	0.279	0.163	0.248
% RSD	0.180	0.106	0.161

Linearity

Standard solutions of derivatized dimethylamine at different concentrations level 8µg/g to 230µg/g were prepared. Calibration curve was constructed by plotting the concentration level of dimethylamine versus corresponding absorbance at 378 nm. The results show an excellent correlation between absorbance and concentration level of dimethylamine within the concentration range (8µg/g to 230µg/g) are given in (Table 4**).** The correlation coefficients were greater than 0.999, which meet the method validation acceptance criteria and hence the method is said to be linear in the range of 8µg/g to 230µg/g

Table 4: Calibration data for dimethylamine standard

n	Dimethylamine standard (µg/g)	Absorbance
1	8.0	0.045
2	15.0	0.091
3	46.0	0.293
4	77.0	0.426
5	154.0	0.898
6	184.0	1.120
7	230.0	1.295
Regression equation		y = 0.005x + 0.012
Correlation coefficient (r^2)		0.999

Accuracy

Accuracy was determined by means of recovery experiments, by the determination of % mean recovery of sample at three different levels (LOQ-150%). At each level, three determinations were performed. Percent mean recovery was calculated as shown in (Table 5). The accepted limits of recovery are 98% - 101% and all observed data are with in the required range which indicates good recovery values and hence the accuracy of the method developed.

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

Accuracy (Average of triplicates)	Level-I (LOQ)	Level-II (100%)	Level-III (150%)
Added(µg/g)	46.65	155.5	233.3
Found(µg/g)	46.48	152.7	230.9
Recovery(%)	99.64	98.05	98.99
RSD(%)	0.76	0.26	0.30

Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It is concluded that the method is robust as it is found that the % RSD is less than 2 for the dimethylamine content despite deliberate variations done concerning compositions of coloured reagent and solvents.

Sensitivity

The sensitivity of measurement of dimethylamine content by use of the proposed method was estimated in terms of the limit of quantitation (LOQ), limit of detection (LOD). The limit of detection (LOD) and limit of quantitiation (LOQ) were found to be 15 µg/g and 46 µg/g respectively. Optical characteristics results are summarized in (Table 6).

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

Parameters	Results
Detection wavelength(nm)	378
Beer's law limits (µg/g)	8.0 – 230
Regression equation (y = mx+c)	y = 0.005x + 0.012
Correlation coefficient (r^2)	0.999
LOQ (µg/g)	46.0
LOD (µg/g)	15.0

CONCLUSION

A cheap and a rapid UV spectrophotometric method was developed and validated for the quantitative estimation of dimethylamine in Metformin HCl drug substance as per ICH guidelines. The developed method resulted in dimethylamine exhibiting linearity in the range 8µg/g to 230µg/g. The interday and intraday precision is exemplified by relative standard deviation of 0.149 % and 0.399%. Percentage Mean recovery was found to be in the range of 98-101%, during accuracy studies. The limit of detection (LOD) and limit of quantitiation (LOQ) were found to be 15 µg/g and 46 µg/g respectively.. Accordingly it is concluded that the developed UV spectrophotometric method is accurate, precise, linear, rugged and robust and therefore the method can be used for the routine analysis of dimethylamine content in Metformin HCl drug substances.

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REFERENCES

[1] Clinical Guidelines Task Force, International Diabetes Federation (**2005**). "Glucose control: oral therapy" PDF (100 KB). In: *Global Guideline for Type 2 Diabetes*. Brussels: International Diabetes Federation, 35–8. Retrieved November 6, **2007**.
[2] National Collaborating Centre for Chronic Conditions. Type 2 diabetes: national clinical guideline for management in primary and secondary care (update) [pdf]. London: Royal College of Physicians; **2008**. ISBN 978-1-86016-333-3. p. 86.
[3] American Diabetes Association. Standards of medical care in diabetes—**2009**. Diabetes Care. **2009**;32 Suppl 1:S13–61. doi:10.2337/dc09-S013. PMID 19118286.
[4] *Diabetes Care*. **2009**;32 Suppl 1:S13–61. doi:10.2337/dc09-S013. PMID 19118286**.**
[5] Joint Formulary Committee (**2013**). "Chapter 6: Endocrine system". *British National Formulary (BNF)* (65 ed.). London, UK: Pharmaceutical Press. pp. 447–448. ISBN 9780857110848.
[6] El Messaoudi, S; Rongen, GA; de Boer, RA; Riksen, NP (December **2011**). *Current Opinion in Lipidology* 22 (6): 445–3.doi:10.1097/MOL.0b013e32834ae1a7. PMID 21897229.
[7] Malek, M; Aghili, R; Emami, Z; Khamseh, ME (**2013**). *ISRN Endocrinology* **2013:** 36927. doi: 10.1155/2013/636927. PMC 3800579. PMID 24224094.
[8] Werner E, Bell J. *J Chem Soc, Transactions*. 1921;121:1790–5.doi:10.1039/CT9222101790.
[9] n-Nitroso-Dimethylamine **CAS #62-75-9** Fact Sheet, Agency for Toxic Substances and Disease Registry (ATSDR), Center for Disease Control (CDC), July **1999** [Online] www.atsdr.cdc.gov/toxfaqs/tfacts141.pdf (accessed Mar 22, **2012).**
[10] International Conference on Harmonization of Technical Requirement for Registration of Pharmaceuticals for Human use. ICH harmonized tripartite guideline. *Validation of analytical procedures text and methodology* Q2 (R1).
[11] M.Rameswara Rao and K. B.Chandrasekhar, *Der Pharma Chemica*, **2011**, 3(2): 358-369.