Determination and validation of hydrazine content by spectrophotometric method in pharmaceutical drug substances

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ABSTRACT

A simple and sensitive spectrophotometric method was developed, optimized and validated for the determination of hydrazine in various drug substances. Which is the process genotoxic impurity. The validation of analytical method was realized trough specificity, linearity, Limit of detection, Limit of quantification, Precision and accuracy parameters. A model compound, Sitagliptin phosphate drug substance was chosen for this study and Limit of detection(LOD) and Limit of quantification(LOQ) values were 0.2 µg/g and 0.6 µg/g respectively. The average accuracy value is 97.8%-100.0%.And also determination of hydrazine in different drug substances like zinc carnosine, aprepitant. In zinc carnosine and aprepitant with slight modification in methodology were discussed in this work. The present work was aimed to develop a visible spectrophotometric method, which is simple, sensitive, accurate and cost effective to evaluate the quality of the bulk drugs.

Keywords: UV Spectrophotometry, Hydrazine, Sitagliptin phosphate, aprepitant,zinc carnosine, Method development, Validation.
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INTRODUCTION

Hydrazine [1,2,3,4] is widely used in pharmaceutical industry as hydrazine hydrate. In applications such as synthesis of intermediate for drug substances. Any impurity other than active moiety is to be controlled with suitable limits in the drug substance irrespective of its harmful nature as per international conference on harmonization(ICH) guideline on impurities, How ever the analysis of hydrazine presents numerous distinctive challenges due to its low molecular weight.Various techniques were employed for the determination of hydrazine in drug substances. Hydrazine is an inorganic compound with chemical formula NH_2-NH_2.It is a colourless flammable liquid with ammonia like odour. Hydrazine [5,6] is a highly toxic and dangerously unstable handled in solution.

The active substance is sitagliptin as monohydrate phosphate salt and its chemical name is chemical name is 7-[(3*R*)-3-amino-1-oxo-4-(2,4,5-trifluorophenyl)butyl]-5,6,7,8-tetrahydro-[3-(trifluoromethyl)-1,2,4-triazolo[4,3-*a*]pyrazine phosphate (1:1) monohydrate according to the IUPAC nomenclature. Sitaglitpin phosphate (Figure. 1) is a white to off white powder and exhibit PH dependent aqueous solubility. The above mentioned active substance contains a chiral centre and is used as a single enantiomer. Structure elucidation has been performed by ultraviolet spectroscopy, Infrared spectroscopy,^{1}H-NMR Spectroscopy,[13]c-NMR spectroscopy and the molecular weight as determined by mass spectroscopy is in agreement with the expected molecular weight the results of X—Ray crystallography are consistent with the proposed molecular structure. Sitagliptin tablets were developed in four strengths 25mg,50mg,100mg and 200mg.It is an oral antihyperglycemic(antibiotic drug).It was developed and marketed by Merck&co. Sitagliptin phosphate monohydrate is a white to off-white crystalline, Nnon hygroscopic

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powder.It is soluble in water and N,N-DMF, Slightly soluble in methanol, very slightly soluble in ethanol,acetone,acetonitrile and insoluble in isopropanol and isopropyl acetate.

Zinc carnosie (Figure. 2) to protect and stabilize the gastric and intestinal mucosal lining. This unique ingradient combines L-Carnosine,a naturally occurring combination of the amino acids beta alanine and L-Histadine with elemental zinc. Zinc carnosine is unique in that it supports the natural protective mechanism in the gastrio intestinal tract with out suppressing stomach acid or other wise interfering in the normal digestive process. Zinc carnosine is a part of our digestive restoration programme which offers you a flexible approach to individualized patient care. Zinc carnosine is a novel dietary ingredient that supports gastrointestinal immune defences, and occasional indigestion. Zinc carnosine is a uniqe combination of the essential mineral zinca, which is a a well known for its antioxidant properties.

Aprepitant (Figure. 3)   is a white to off-white crystalline solid and practically insoluble in water. Aprepitant hasthe chemical name 5-[[(2R,3S)-2-[(1R)-1-[3,5bis(trifluoromethyl)phenyl]ethoxy]-3-(4-fluorophenyl)-4morpholinyl]methyl]-1,2-dihydro-3H-1,2,4-triazol-3-one.

Two crystalline forms exist but only one form (Form I), which is the most thermodynamically stable Two crystalline forms exist but only one form which is the most thermodynamically stable polymorph, is produced and used in the drug product. satisifactory conformance has been provided that are is not any conversion to undesirable form. Aprepitatnt is a chiral, containing 3 asymmetric centres. Aprepitant is a whit to off white crystalline poder with molecular weight 534.43.It is practically insoluble in water. It is sparingly soluble in ethanol and isopropyl acetate. and slightly soluble in acetonitrile. EMEND capsules contain the active ingredient, aprepitant. Each capsules of EMEND for oral administration contains either 40mg,80mg or 125mg of a prepitant and the following inactive ingredients. Sucrose, micro crystalline cellulose.

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 drugs Sitagliptin phosphate, Zinc carnosine, Aprepitant 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.

Methods
Preparation of Solutions:
 Reagent solution: Dissolve about 0.8g of 4-Dimehtyl amino benzaldehyde in a mixture of 40 ml of ethanol and 4 ml of concentrate hydrochloric acid.

Standard solution: Accurately weighed and transferred about 0.2041g of hydrazine sulphate into a clean 100 ml volumetric flask dissolve in and dilute to volume with water. Pipette out 1 ml of above solution into 100 ml volumetric flask and make up to volume with methanol to give 1ml equals to 5µg hydrazine. From this dilute 10 ml of this solution to 100 ml to get a concentration 0.5µg.

Sample solution:
Sitagliptin phosphate: In a 25 ml volumetric flask add about 0.66 g of sitagliptin phosphate add 10 ml water sonicate to dissolve the sample completely then add 10 ml colour reagent make up to the volume with 1M Hydrochloric acid.
Zinc carnosine: In a 25 ml volumetric flask add about 1.0 g of Zinc carnosine add 1 ml of concentrated nitric acid sonicate to dissolve and add 10 ml colour reagent make up to the volume with 1M Hydrochloric acid.

Aprepitant: In a25 ml volumetric flask add about 1.0 g of Aprepitant add 10 ml of 96% ethanol sonicate to suspend it if still it is hazy add 10 ml colour reagent then it will clear make up with 96% Ethanol to dissolve and add 10 ml colour reagent make up to the volume with 1M Hydrochloric acid.

Blank preparation: Prepare as mentioned in sample preparation with out sample addition. and make necessary correction for all samples.

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Procedure:

Keep all the above solutions aside for 15 mins. then filter through 0.45µ membrane filter(if required).Record the absorbance of standard and sample solutions at 458 nm by applying blank correction. The developed yellow coloured complex of standard and sample solution scanned between 400 nm to 600 nm and the absorbance maxima at λ 458nm.

Spectral Characteristics: Standard solutions of derivatized hydrazine at different concentrations level were prepared. Calibration curve was constructed by plotting the concentration level versus corresponding absorbance at 458nm. The results show an excellent correlation between absorbance and concentration level of hydrazine within the concentration range 0.2 to 27.0 µg/g show good agreement with Beer's law.

RESULTS AND DISCUSSIONS

Method development

The colour reagent consist the following reagents P-Dimehtyl amino benzaldehyde, water and concentrated hydrochloric acid. At room temperature the yellow colour develops immediately and it is stable for some time. (Figure. 4) The intensity of the colour of the solution is proportional to the hydrazine concentration. this is the basis to determine the content of hydrazine. We measured the hydrazine content in drug substance low levels. (Figure 5) On the basis of trail and error method colour reagent is prepared. The intensity of the color of the aqueous solution is proportional to the hydrazine concentration. (Figure. 6). This is the basis to determine the content of free hydrazine[7,8,9]. We measured the hydrazine content in drug substance low levels.

Method validation

Validation of the analytical method is the process that establishes by laboratory studies in which the performance characteristics of the method meet the requirements for the intended analytical application. UV spectrophotometric method developed was validated according to International Conference on Harmonization (ICH) guidelines[10] for validation of analytical procedures. The method was validated for the parameters like linearity, accuracy, system precision, intra-day precision, inter-day precision/ intermediate precision/ ruggedness, robustness, limit of detection (LOD) and limit of quantitiation (LOQ).

Precision
System precision

Six replicate recording of absorbance at 458 nm of standard solution at working concentration showed % RSD (Relative Standard Deviation) less than 1 concerning absorbance for the hydrazine, which indicates the acceptable reproducibility and thereby the precision of the system. System precision results are tabulated in (Table 1).

Method precision

Method precision was determined by performing content of hydrazine by spiking with known concentration of hydrazine in different drug substances under the test of repeatability (Intra day precision)performed.

Repeatability (Intra day precision)

Six consecutive recording of absorbance at 458 nm of the Hydrazine 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, 3 and 4).

Linearity

Standard solutions of derivatized hydrazine at different concentrations level 0.2 to 27 µg/g were prepared. Calibration curve was constructed by plotting the concentration level of hydrazine versus corresponding absorbance at 458 nm. The results show an excellent correlation between absorbance and concentration level of hydrazine within the concentration range (0.2-27.0 µg/g) are given in 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 0.2-27.0 µg/g. (Table 5)

LOD and LOQ (Limit of detection and Limit of quantification):

For determining the limit of detection(LOD) and Limit of quantification (LOQ),The method based on the residual standard deviation of a regression line and slope was found to be LOD is 0.3µg/g and LOQ is 0.9µg/g. But actual response of hydrazine we chosen LOD is 0.2µg/g and LOQ is 0.6µg/g and it is verified by preparing LOD,LOQ precision experiment. the RSD Of LOD is less than 33% and LOQ Is 10%.The LOD and LOQ Solutions were prepared at about predicted concentration levels and analyzed six times for checking precision.

LOD and LOQ Precision:
The LOD and LOQ solutions were prepared at predicted concentration and analysed as per the methodology. the RSD was found to be less than 10 and 33%(Table 6 and 7).

Accuracy:
Accuracy of the method was performed by recovery experiments using standard addition technique. The recoveries of I,II and III were determine by spiking hydrazine at three different levels into 3 different drug substances these samples were prepared as per the procedure and analysed in triplicate and the percentage recoveries were calculated the recovery value for hydrazine ranged from 95-102%.The fully validated accuracy results were shown in the table.(Table 8,9 and 10)

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 formaldehyde content despite deliberate variations done concerning compositions of colored reagent and solvents.

Sensitivity
The sensitivity of measurement of hydrazine 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 0.20 µg/g and 0.60 µg/g respectively. Optical characteristics results are summarized in (Table 11)

Figure. 1: Chemical Structure of Sitagliptin phosphate

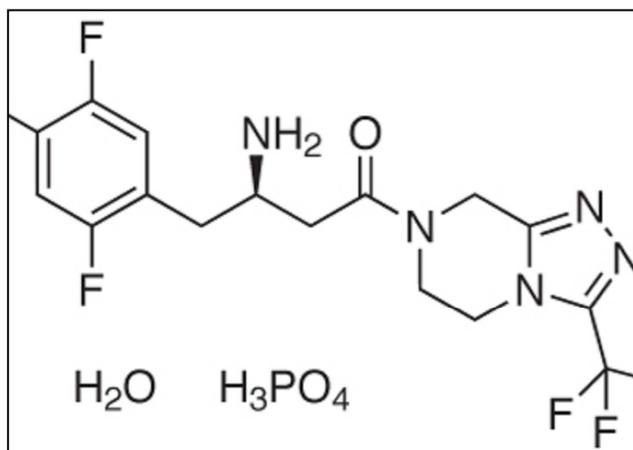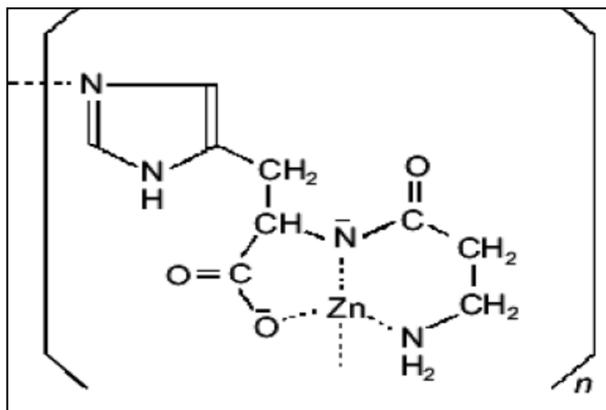**Figure. 2: Chemical Structure of Zinc carnosine**

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Figure. 3: Chemical Structure of Aprepitant
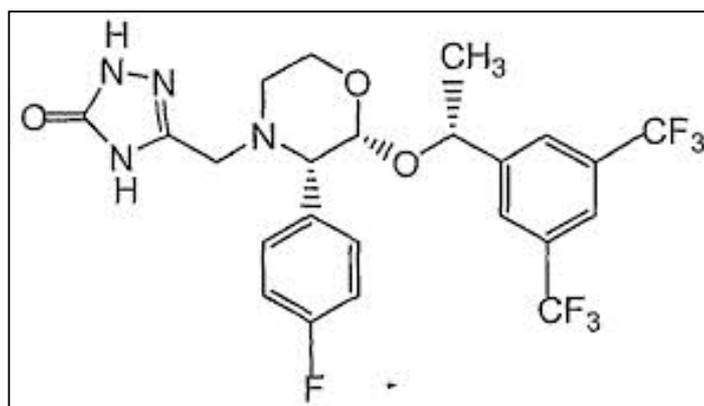

Figure. 4: Basic chemical reaction involved for colour development
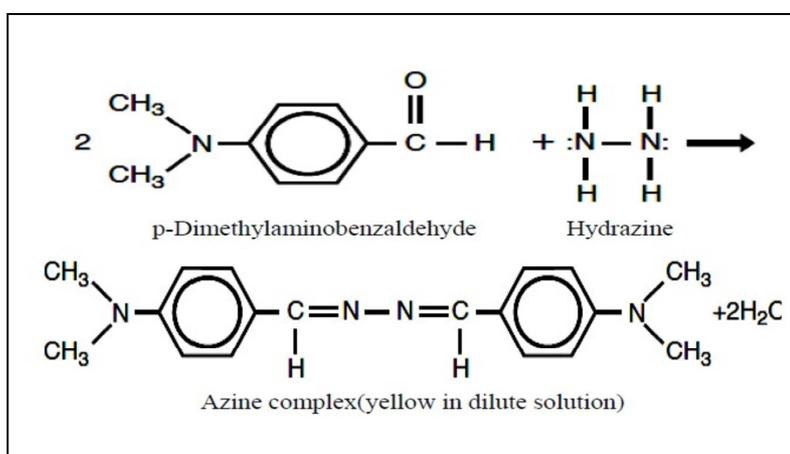

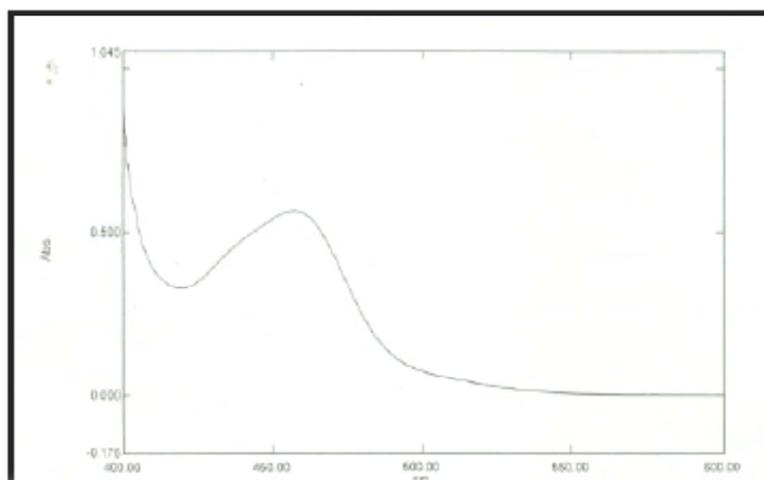**Figure. 5: UV Spectrum of derivatised Hydrazine**

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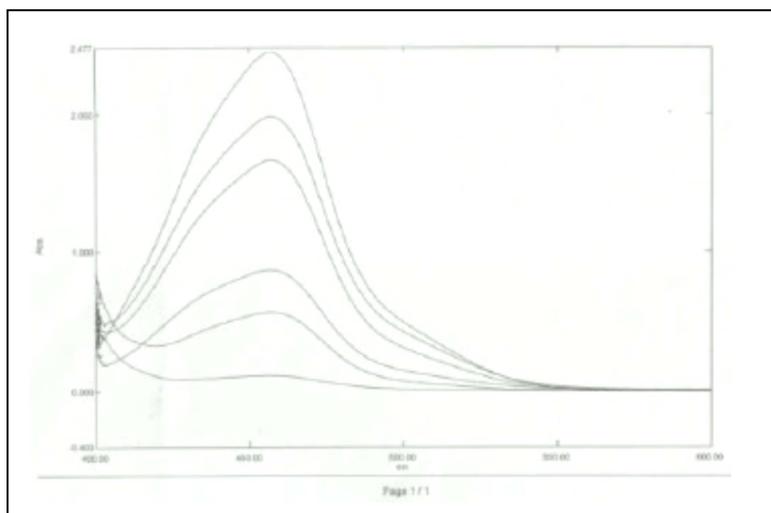**Figure 6: Overlay spectrum of derivatised hydrazine at different concentrations**

Table 1.: System precision results of Hydrazine

n	Absorbance
1	0.857
2	0.859
3	0.856
4	0.856
5	0.856
6	0.857
Mean	0.857
SD^	0.001
%RSD*	0.1

^ Standard deviation
* Relative standard deviation

Table 2.: Intra day precision results of Hydrazine(15.0 µg/g)spiked in Sitagliptin phosphate drug substance

n	Hydrazine Content(µg/g)
1	15.03
2	15.07
3	15.17
4	15.15
5	15.12
6	15.28
Mean	15.14
SD^	0.087
%RSD	0.6

Table 3.: Intra day precision results of Hydrazine(10.0 µg/g)spiked in Zinc carnosine drug substance

n	Hydrazine Content(µg/g)
1	9.94
2	9.93
3	10.02
4	10.03
5	10.01
6	10.03
Mean	9.99
SD^	0.046
%RSD	0.5

Table 4.: Intra day precision results of Hydrazine(10.0 μg/g)spiked in Aprepitant drug substance

n	Hydrazine Content(μg/g)
1	9.95
2	9.92
3	10.08
4	9.98
5	9.95
6	9.96
Mean	9.97
SD^	0.056
%RSD	0.6

Table 5: Calibration data for Hydrazine standard

n	Hydrazine standard (μg/g)	Absorbance
1	0.20	0.032
2	0.60	0.044
3	0.80	0.068
4	1.00	0.083
5	5.00	0.415
6	10.00	0.855
7	15.00	1.275
8	18.00	1.525
9	22.00	1.866
10	27.00	2.281
Regression equation		y = 0.084x +0.001
Correlation coefficient (r^2)		0.999

Table 6.: Limit of Detection for precision

S.no	Absorbance
1	0.022
2	0.019
3	0.018
4	0.023
5	0.016
6	0.022
Mean	0.020
SD^	0.003
%RSD	15.0

Table 7.: Limit of Quantification for precision

S.no	Absorbance
1	0.048
2	0.049
3	0.046
4	0.050
5	0.049
6	0.048
Mean	0.048
SD^	0.001
%RSD	2.10

Table 8.: Recovery results from spiking of Sitagliptin phosphate drug substance with Hydrazine

Accuracy (Average of triplicates)	Level-I (LOQ)	Level-II (100%)	Level-III (150%)
Added(μg/g)	0.90	15.08	22.58
Found(μg/g)	0.90	14.83	22.29
Recovery(%)	100.0	98.30	98.7
RSD(%)	0.60	0.01	1.70

Table 9.: Recovery results from spiking of Zinc carnosine drug substance with Hydrazine

Accuracy (Average of triplicates)	Level-I (LOQ)	Level-II (100%)	Level-III (150%)
Added(µg/g)	0.60	9.94	14.92
Found(µg/g)	0.58	9.86	14.59
Recovery(%)	96.7	99.2	98.7
RSD(%)	1.70	0.01	0.70

Table 10.: Recovery results from spiking of Aprepitant drug substance with Hydrazine

Accuracy (Average of triplicates)	Level-I (LOQ)	Level-II (100%)	Level-III (150%)
Added(µg/g)	0.60	9.96	14.96
Found(µg/g)	0.58	9.98	14.87
Recovery(%)	97.8	100.2	99.4
RSD(%)	0.90	0.01	0.4

Table 11.: Optical characteristics of Hydrazine content in pharmaceutical drug substances

Parameters	Results
Detection wavelength(nm)	458
Beer's law limits (µg/g)	0.2 – 27
Regression equation (y = mx+c)	y = 0.084x+0.001
Correlation coefficient (r^2)	0.999
LOQ (µg/g)	0.20
LOD (µg/g)	0.60

CONCLUSION

A cheap and a rapid UV spectrophotometric method was developed and validated for the quantitative estimation of Hydrazine in pharmaceutical drug substances as per ICH guidelines. The developed method resulted in hydrazine exhibiting linearity in the range 0.2 µg/g to 27µg/g. The system precision of hydrazine standard RSD was found to be 0.1%. Intra day precision result of hydrazine in pharmaceutical drug substances was found to be 0.6,0.5 and 0.6% in Sitaglitpin phosphate, Zinc carnosine and aprepitant respectively. Percentage Mean recovery was found to be in the range of 97.8 -100.2%, during accuracy studies in different drug substances. The limit of detection (LOD) and limit of quantitiation (LOQ) were found to be 0.20 µg/g and 0.60 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 hydrazine content in different pharmaceutical drug substances.

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