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Evaluation and comparison of expired and extant metformin drug by method validation using UV-VIS spectrophotometry

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ABSTRACT

Metformin, a widely used antihyperglycemic drug for the treatment of type 2 diabetes mellitus. The usage of this therapeutic drug has become more common as the frequency of diabetes mellitus metabolic disorder is high worldwide especially in India. Improper disposal of the expired drug is creating a huge impact in the environment as well as financial prudence for the country. UV-visible spectrophotometry is one of the simple and accurate method for the apposite validation of drug. Our aim is to evaluate and compare the expired and extant metformin drug by UV-visible spectrophotometry. We observed the absorption maxima at 233nm and the resolution of the analyte peak is highly specific for metformin drug. The calibration curve with different concentration of both expired and extant drugs was found to be linear. The repeated interday and intraday measurements of both the drug at 10 ppm concentration were extremely precise and the percentage recovery was accurate by zero order spectroscopy. The drug solution was stable at different time points and the robustness during all variations was not much affected for both the expired and extant metformin drug. We observed the system suitability is satisfactory and fulfilled all the parameters of method validation. The detection and quantification limit of both the drugs was found to be the lowest concentration about 1ppm as an exact value. Our results conclude that there is no significant variation in all the method validation parameters between the 10 years expired and extant metformin drug. This will be helpful to avoid wastage or disposal of enormous expired metformin drug and to reconsider the extension of expiry date by pharmaceutical companies with proper approval of the organizations.

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INTRODUCTION

Metformin, a biguanide hypoglycemic agent widely used for the treatment of non-insulin dependent type 2 diabetes mellitus for lowering the blood glucose levels. Several studies reported that metformin also has a potential role in anti-aging, antitumour, cardioprotective and neuroprotective effect (Wang et al., 2017). Metformin has various other benefits in patients with diabetes mellitus such as decrease in hyperinsulinemia, reduction of weight, improvement in lipid profiles, augmented fibrinolysis, and

increased endothelial function (Nasri and Rafieian-Kopaei, 2014). The therapeutic effect of metformin will be achieved by, without an increase in insulin concentration and also reduce insulin resistance (Mahadik *et al.*, 2012). Metformin targets multiple pathways, including antioxidation, angiogenesis, inflammation, autophagy and cellular survival (Wang *et al.*, 2017). The major mechanism of action of metformin includes reduction of appetite, diminished carbohydrate absorption in the intestine, inhibition of gluconeogenesis in hepatic system and enhanced uptake of glucose by peripheral tissues. The efficacy of the drug is well documented in most of the studies in the form of monotherapy and combination therapy as highly safety, cost effective and an excellent choice in both specialized treatments and primary health care (Papanas and Maltezos, 2009).

Method validation is an analytical process essential for the confirmation of valid measurements used to assure the reliability, quality and consistency of results which is an important factor of good analytical practice. Several international organizations refer method validation guidelines such as European cooperation of Accreditation (EA), European Committee for Normalization (CEN), Codex Committee on Methods of Analysis and Sampling (CCMAS), Cooperation on International Traceability in Analytical Chemistry (CITAC), American Society for Testing and Material (ASTM), Unites States Food and Drug Administration (FDA), Food and Agricultural Organization (FAO) and International Conference on Harmonization (ICH). The four major types of method validation related analytical procedures were categorized as identification of suitable tests, control of impurity tests, qualitative tests and quantitative tests for the samples, drug substances, components of drugs and drug products. The validation also includes and necessary to assess the modifications in synthesis of drug substance and composition of drug products. The authors suggest that UV-Visible spectrophotometric method is suitable for validation of drug as per ICH Q2(R1) guideline (Behera *et al.*, 2012). Mahadik et al. described and developed suggested that UV spectroscopic method is simple, sensitive, specific, accurate, cost effective and precise method for bulk estimation of metformin in tablet formulation (Mahadik *et al.*, 2012). The significance of the study was planned to assess the further stability of the drug beyond its expiration date and to instigate the manufactures to register for shelf-life extension of this drug from Central Drugs Standard Control Organisation (CDSCO). This study outcome might help to refine our prescription drug expiration process and to alleviate the economic burden

of procuring the medicines frequently by the consumers. Based on the background our aim is to evaluate and compare the expired and extant metformin drug using UV-Visible spectrophotometry.

MATERIALS AND METHODS

Drugs used for validation

Metformin Hydrochloride tablets - 500mg (Melmet-500) from micro labs was used in this study. The ten years expired metformin drug (EXP: June 2012, B.No. MEAD0033) and the extant metformin drug currently in usage (EXP: June 2022, B.No. MEAS0033) was utilized for comparative analysis and validation of study parameters. Analytical grade methanol and water were utilised for the experiments. Methanol and Water (15:85, v/v) used as diluent to dissolve and prepare the required concentration of the drug. 10mg drug was dissolved in 10ml of diluent to attain 1000 ppm. From this 10 microliter make up to 1000 microliter to get 10 ppm solution.

Method validation parameters

The method validation of ICH guidelines was followed for evaluation and comparison of expired and extant metformin drugs. Validation of drugs was done using UV-VIS Spectrophotometer (Hitachi – UH5300-3J1-0015). The drug validation parameters are selection of wavelength, specificity, linearity, precision, accuracy, solution stability, robustness, system suitability, limit of detection and limit of quantification.

RESULTS AND DISCUSSION

Selection of analytical wavelength and absorption maxima

Drug solutions were measured in the wavelength range of 200-400 nm in UV double beam spectrophotometry. Instrument set at zero and the baseline correction was done (Figure 1). Then the diluent (Figure 2), expired drug (Figure 3) and extant drug (Figure 4) was measured at 200-400 nm to assess the exact absorption maxima. The zero order absorption spectra obtained was selected for the analysis of the drugs. The absorption maximum was found at 233 nm, which is used for further analysis.

Specificity

The resolution of the analyte peak was measured from the nearest peak obtained by repetitive analysis of the analyte using the same solution and settings.

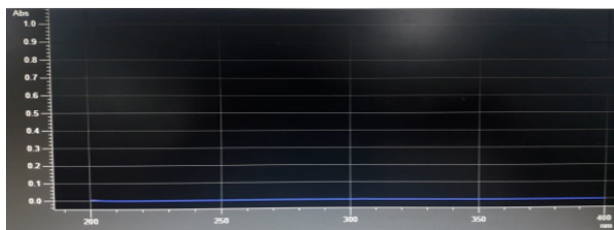The 10 ppm concentration of the drug was measured multiple times in the range of 200-400nm.

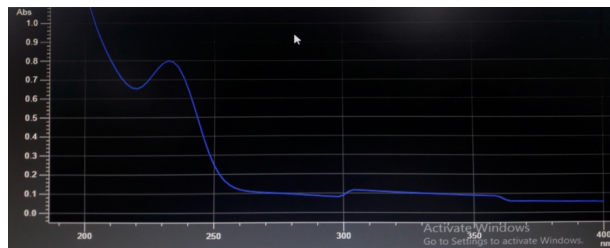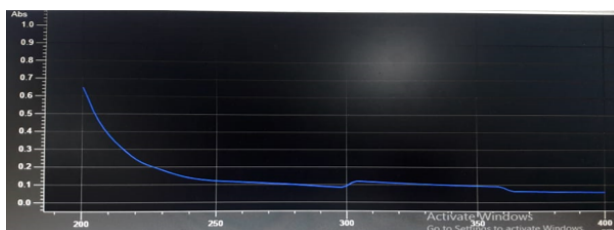**Figure 1: Baseline correction in UV-Visible spectrophotometry**

Figure 2: Analytical wavelength of diluent

Figure 3: Analytical wavelength and absorption maxima 233nm of expired drug

Figure 4: Analytical wavelength and absorption maxima 233nm of extant drug

We observed the peak falls in the same position at 233nm for both expired (Figure 5) and extant (Figure 6) metformin drug. This indicates that the resolution of the analyte peak is specific at 233nm for metformin.
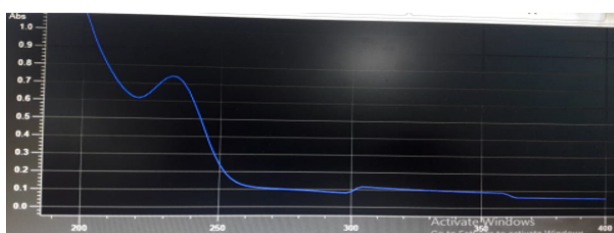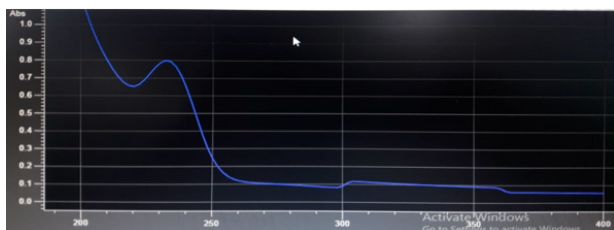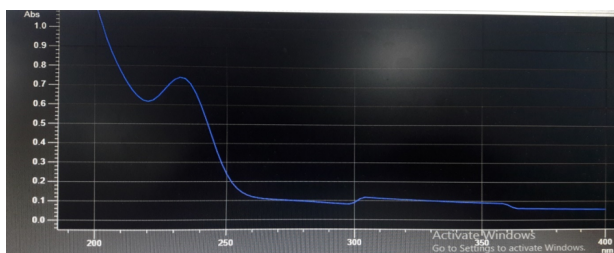

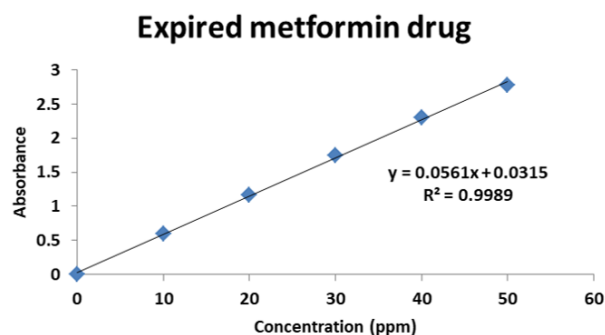**Figure 5: Analytical wavelength and absorption maxima 233nm of expired drug**

Figure 6: Analytical wavelength and absorption maxima 233nm of extant drug

Figure 7: Linear calibration curve of expired metformin drug
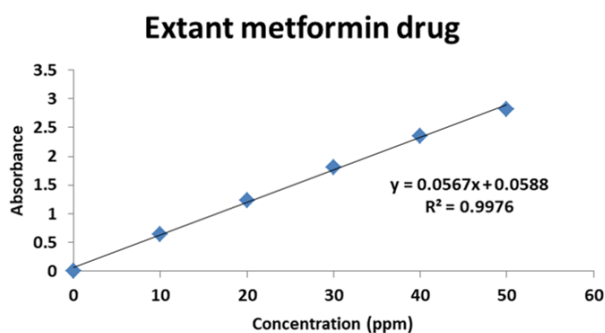
Figure 8: Linear calibration curve of extant metformin drug

Linearity

The absorbance was measured at 233nm with the concentration range from 1 – 50 ppm for both the expired and extant metformin drug (Table 1). Calibration curve was obtained and the response of the drug was found to be linear in the investigated concentrations for both the expired and extant metformin. The linear regression equation for expired drug was y=0.0561x+0.0315 with correlation coefficient 0.9989 and for extant drug was y=0.0567x+0.0588 with correlation coefficient 0.9976 (Figures 7 and 8).

Precision

The precision of the analytical method was carried out using 10 ppm concentration of the drug and repeated the measurement at 233nm six times intraday and interday for both the expired and extant

Table 1: Linearity of expired and extant metformin drug

Linearity of expired drug		Linearity of extant drug	
Concentration (ppm)	Absorbance (233 nm)	Concentration (ppm)	Absorbance (233 nm)
0	0.000	0	0.000
10	0.598	10	0.640
20	1.165	20	1.226
30	1.746	30	1.815
40	2.307	40	2.353
50	2.783	50	2.823

Table 2: Precision of expired and extant metformin drug

Precision of expired drug			Precision of extant drug		
Set	Intraday	Interday	Set	Intraday	Interday
1	0.595	0.538	1	0.609	0.57
2	0.595	0.538	2	0.609	0.57
3	0.595	0.538	3	0.609	0.57
4	0.595	0.538	4	0.609	0.57
5	0.595	0.538	5	0.609	0.57
6	0.595	0.538	6	0.609	0.57
Mean	0.595	0.538	Mean	0.609	0.57
SD	0	0	SD	0	0
%RSD	0	0	%RSD	0	0

Table 3: Accuracy of expired metformin drug

	Accuracy of expired metformin drug by zero order spectroscopy			
Drug Solution	Constant amount (ppm)	Added amount (ppm)	Drug Recovered (ppm)	% Recovery $\pm$ SD
50%	20	10	10	100 $\pm$ 0.00
100%	20	20	20	100 $\pm$ 0.00
150%	20	30	30	100 $\pm$ 0.00

Table 4: Accuracy of extant metformin drug

	Accuracy of extant metformin drug by zero order spectroscopy			
Drug Solution	Constant amount (ppm)	Added amount (ppm)	Drug Recovered (ppm)	% Recovery $\pm$ SD
50%	20	10	10	100 $\pm$ 0.00
100%	20	20	20	100 $\pm$ 0.00
150%	20	30	30	100 $\pm$ 0.00

Table 5: Solution stability of expired and extant metformin drug

Time (Hrs.)	Expired drug (233nm)	Extant drug (233nm)
0	0.598	0.640
1	0.595	0.638
2	0.584	0.627
3	0.571	0.609

Table 6: Robustness of expired and extant metformin drug

	Robustness of expired drug				Robustness of extant drug		
S.no	232nm	233nm	234nm	S.no	232nm	233nm	234nm
1	0.585	0.595	0.568	1	0.608	0.609	0.595
2	0.586	0.595	0.568	2	0.608	0.609	0.595
3	0.587	0.595	0.568	3	0.608	0.609	0.595
4	0.587	0.595	0.567	4	0.608	0.609	0.595
5	0.584	0.595	0.567	5	0.608	0.609	0.596
6	0.584	0.595	0.567	6	0.608	0.609	0.596
Mean	0.5855	0.595	0.5675	Mean	0.608	0.609	0.595333
SD	0.001378	0	0.000548	SD	0	0	0.000516
%RSD	0.235424	0	0.096515	%RSD	0	0	0.086741

Table 7: System suitability of expired and extant metformin drug

S.no	Expired drug (233nm)	Extant drug (233nm)
1	0.595	0.609
2	0.595	0.609
3	0.595	0.609
4	0.595	0.609
5	0.595	0.609
6	0.595	0.609
Mean	0.595	0.609
SD	0	0
%RSD	0	0

Table 8: Detection limit of expired and extant metformin drug

Concentration (ppm)	Expired drug absorbance (233 nm)	Extant drug absorbance (233 nm)
1	0.045	0.016
10	0.597	0.640
20	1.165	1.226
30	1.745	1.815
40	2.307	2.352
50	2.786	2.825

metformin. The mean value, standard deviation and percentage of relative standard deviation were calculated. We observed the developed method was found to be precise as %RSD of repeatability and intermediate values for both the drug is zero (Table 2).

Accuracy

The accuracy of the proposed method was determined by adding different amounts of drug like 50%, 100% and 150% of metformin within linearity range and added to the pre-analysed constant concentration and percentage recovery was calculated. The accuracy was obtained for zero order spectroscopy was found to be present within the range (Tables 3 and 4).

Solution stability

The solution stability was measured at different time points. It was found that the drug solution prepared for analysis is stable at room temperature and the result was not decreased below the minimum measurable levels. This indicates the drug solution used for the method validation was stable during the experiment at room temperature (Table 5).

Robustness

The robustness evaluation of the drug should be considered for reliability of analysis with respect to the deliberate variations in the developed method. We observed only very subtle considerable differ-

ence in the mean values, whereas the standard deviation and %RSD was found to be similar as zero for both the tested drugs. The robustness of the drug solution during all variations were not affected much and it was measured the actual values (Table 6)

System suitability

The system suitability test of spectrophotometric system should be performed before each validation to assess the suitability of the instrument and the method. The analyte was measured at 233nm for six times and the %RSD was calculated which should not be more than 2.0% for acceptance of system suitability. We observed the %RSD for both the metformin drug is zero, indicating that the system suitability is satisfactory and full filled all the parameters of method validation (Table 7).

Limit of detection

The limit of detection of an analytical procedure indicates the lowest amount of an analyte in a sample that can be detected by the instrument and developed method. The detection limit was determined by preparing different concentration range from 1-50 ppm of drug of the absorbance was measured at 233nm in spectrophotometry. The lowest concentration absorbance of expired drug was 0.045 and extant drug was 0.015. We observed for both the drugs the lowest concentration about 1ppm can be detected and quantified as an exact value (Table 8).

Limit of quantification

The limit of quantification is the reliable concentration quantitated with specified accuracy and precision. The quantification limit calculated using a formula with a standard deviation of drug response and the slope of the calibration curve. Mostly the limit of detection is equal to the limit of quantification. We observed the accuracy and precision validation data was perfect for the proposed method. With these observations, the limit of quantification in both the drugs was found to be 1 ppm or 1.2 μg/ml in spectrophotometric method.

CONCLUSION

We designed our study with the concern of avoiding disposal of enormous expired drugs and to evaluate the shelf life of the drug to extend its usage. Our study results conclude that there is no major difference in the validation between 10 years expired and extant metformin drug. This indicates that the shelf life of the expired drugs is extended to usage even after 10 years specific for metformin drug. We also observed that UV-VIS spectrometry is simple, specific, sensitive, linear, accurate and precise technique for the method validation and evaluation of the drugs. Our results will be helpful for consideration of extension of the expiry date of metformin drug by the regulatory bodies. This will be highly beneficial for the society as frequency of purchase, economical, extensive usage and effective drug for long term users of metformin.

Conflict of Interest

The authors declare that there is no conflict of interest for this study.

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