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EVALUATION OF LAWSONE AS AN ANALYTICAL REAGENT FOR COLORIMETRIC ESTIMATION OF ACTIVE PHARMACEUTICAL INGREDIENTS

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1. ABSTRACT

The aim of the present work is to estimate various API having different functional groups using Lawsone as a derivatizing agent. As lawsone is used in various herbal formulations as a hair colouring agent, hence it can be established as a tool to develop the analytical methods for estimation of various drugs. Numerous formulations in the market are available with API's having no absorbance in the visible region (400-800 nm). Estimation of some of them is made possible in visible region by reacting it with Lawsone in different experimental conditions. Hence, we can estimate the drugs that are devoid of chromophores using Lawsone reagent. Simple colorimetric method is used for estimation and the method is clear, precise and cheap. We applied lawsone as a derivatizing agent for derivatization of different pharmaceuticals, which was used for the estimation of them. Linearity was taken after derivatization with Lawsone and absorbance was measured at 482 nm for Sulphanilamide, 496 nm for Ramipril, 433 nm for Vanillin, 453 nm for Diclofenac, 455 nm for Glycine, 435 nm for Dextrose, 455 nm for L-Glutathione reduced, 454 nm for Metformin. Validation of developed methods was performed according to ICH Q2R1guideline.

2. KEYWORDS: Active Pharmaceutical Ingredient (API), Lawsone, Derivatizing agent, Colorimetric method.

3. INTRODUCTION

Lawsone (2-hydroxy-1, 4-naphthoquinone) also known as hennotannic acid, is a red-orange dye present in the leaves of the henna plant.

Biological Source: Lawsone is extracted from plant of Lawsonia inermis or Henna (commonly known as mehndi plant) belonging to the family **Lythraceae.**[1]

Humans have used henna extracts containing lawsone as hair and skin dyes for more than 5000 years. Lawsone reacts chemically with the protein known as keratin in skin and hair, in a process known as Michael addition, resulting in a strong permanent stain that lasts until the skin or hair is shed. The darker coloured ink is due to more lawsone-keratin interactions occurring, which evidently break down as the concentration of lawsone decreases and the tattoo fades.[2]

Lawsone strongly absorbs UV light, and aqueous extracts can be effective for sunless tanning and in sunscreens. Chemically, lawsone is similar to juglone, which is found in walnuts.[3]

Lawsone is having various applications like Abortifacient activity, Hepatoprotective activity, Anti-microbial activity, Anti-Helminthic activity, Anti-Trypanosome, Anti-fungal activity, staining reagent for gram positive bacteria, Immunomodulatory effect, Anti-oxidant activity, and Anti-cancer activity.[4]

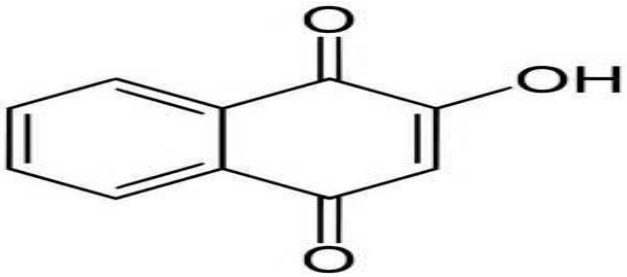


Fig. 1: 2-hydroxy-1, 4-naphthoquinone (Lawsone).[1]

4. MATERIALS AND METHODS

Materials: API of Sulphanilamide, Ramipril, Vanillin, Diclofenac, Glycine, Dextrose, L-Glutathione reduced, Metformin were procured from Oxford chemicals and Jenburkt pharmaceuticals. The API of Lawsone was obtained from Yukka enterprise. Finer grade of

methanol and ethanol were used and Sartorius Filter Paper 0.2 microns (Sartorius, Germany). Also, p-Nitro aniline, Formaldehyde-AR, sodium hydroxide (Finer Reagent) were used.

Instrumentation: Instruments used in the study were UV-visible spectrophotometer (Shimadzu 1800- Double beam spectrophotometer) with Quartz cuvette pair with 1 cm path length at 800 – 200 nm scanning range.

Experimental solutions

5.1 Sulphanilamide

❖ **Preparation of 5N Hydrochloric acid solution:** Accurately measured volume of 42.5 ml concentrated Hydrochloric acid was transferred into a 100 ml volumetric flask then diluted up to mark with distilled water to obtain final concentration 5N Hydrochloric acid solution.

❖ **Preparation of 0.2% w/v Sodium nirate solution:** Accurately weighed quantity of 0.2 g of Sodium nirate was transferred into 100 ml volumetric flask, then diluted up to mark with distilled water to obtain final concentration 0.2% w/v Sodium nitrate solution.

❖ **Preparation of 0.5% w/v Ammonium sulphamate solution:** Accurately weighed quantity of 0.5 g of Ammonium sulphamate was transferred into 100 ml volumetric flask then diluted up to mark with distilled water to obtain final concentration 0.5% w/v Ammonium sulphamate solution.

❖ **Preparation of Lawsone solution:** Accurately weighed quantity of 50 mg of Lawsone was transferred into 50 ml volumetric flask then diluted up to mark with distilled water to obtain final concentration 0.1% w/v Lawsone solution.

❖ **Preparation of working standard solution of sulphanilamide:** Accurately weighed quantity of 10 mg of sulphanilamide was transferred into 10 ml volumetric flask then diluted up to mark with distilled water to obtain final concentration 1000 µg/ml of sulphanilamide solution.

❖ **Preparation of calibration curve for sulphanilamide:** In a series of 5 different 10 ml volumetric flasks 0.2 ml, 0.3 ml, 0.4 ml, 0.5 ml and 0.6 ml working standard solution of sulphanilamide were transferred. 3.5 ml 5N HCl solution, 0.5 ml Sodium nirate solution, 0.5 ml Ammonium sulphamate solution, 0.5 ml 0.1% solution of Lawsone were added in each flasks. Volumes were made up with distilled water. Spectrum of prepared standard solutions having concentration 20, 30, 40, 50 and 60 µg/ml have been recorded between 400 to 800 nm

using UV-Visible spectrophotometer taking reagent blank at λmax 482 nm. Absorbance was plotted against concentration and the regression equation was determined.

5.2 Ramipril

❖ **Preparation of 5N Hydrochloric acid solution:** Accurately measured volume of 42.5 ml concentrated Hydrochloric acid was transferred into a 100 ml volumetric flask then diluted up to mark with distilled water to obtain final concentration 5N Hydrochloric acid solution.

❖ **Preparation of 0.2% w/v Sodium nirate solution:** Accurately weighed quantity of 0.2 g of Sodium nirate was transferred into 100 ml volumetric flask, then diluted up to mark with distilled water to obtain final concentration 0.2% w/v Sodium nitrate solution.

❖ **Preparation of 0.5% w/v Ammonium sulphamate solution:** Accurately weighed quantity of 0.5 g of Ammonium sulphamate was transferred into 100 ml volumetric flask then diluted up to mark with distilled water to obtain final concentration 0.5% w/v Ammonium sulphamate solution.

❖ **Preparation of Lawsone solution:** Accurately weighed quantity of 50 mg of Lawsone was transferred into 50 ml volumetric flask then diluted up to mark with distilled water to obtain final concentration 0.1% w/v Lawsone solution.

❖ **Preparation of working standard solution of Ramipril:** Accurately weighed quantity of 10 mg of ramipril was transferred into 10 ml volumetric flask then diluted up to mark with distilled water to obtain final concentration 1000 µg/ml of Ramipril.

❖ **Preparation of calibration curve for Ramipril:** In a series of 6 different 10 ml volumetric flasks 0.2 ml, 0.3 ml, 0.4 ml, 0.5 ml, 0.6 ml and 0.7 ml working standard solution of Ramipril were transferred. 3.5 ml 5N HCl solution, 0.5 ml Sodium nirate solution, 0.5 ml Ammonium sulphamate solution, 0.5 ml 0.1% solution of Lawsone were added in each flasks. Volumes were made up with distilled water. Spectrum of prepared standard solutions having concentration 20, 30, 40, 50, 60 and 70 µg/ml have been recorded between 400 to 800 nm using UV-Visible spectrophotometer taking reagent blank at λmax 496 nm. Absorbance was plotted against concentration and the regression equation was determined.

5.3 Vanillin

❖ **Preparation of 0.1% w/v Lawsone solution:** Accurately weighed quantity of 50 mg of Lawsone was transferred into 50 ml volumetric flask then diluted up to mark with ethanol to obtain final concentration 0.1% w/v Lawsone solution.

❖ **Preparation of p-Nitro aniline solution:** Accurately weighed quantity of 100 mg of p-nitro aniline was transferred into 10 ml volumetric flask then diluted up to mark with ethanol to obtain 1% w/v p-Nitro aniline solution.

❖ **Preparation of working standard solution of vanillin:** Accurately weighed quantity of 10 mg of vanillin was transferred into 10 ml volumetric flask then diluted up to mark with distilled water to obtain final concentration 1000 µg/ml of vanillin.

❖ **Preparation of calibration curve for vanillin:** In a series of 5 different 10 ml volumetric flasks 0.2 ml, 0.3 ml, 0.4 ml, 0.5 ml and 0.6 ml working standard solution of vanillin were transferred. 2 ml 0.1% Lawsone solution, 0.6 ml 1% w/v p-Nitro aniline solution were added in each flasks. Volumes were made up with ethanol. Spectrum of prepared standard solutions having concentration 20, 30, 40, 50 and 60 µg/ml have been recorded between 400 to 800 nm at λmax 433 nm taking reagent blank using UV-Visible spectrophotometer. Absorbance was plotted against concentration and the regression equation was determined.

5.4 Diclofenac

❖ **Preparation of 0.1% w/v Lawsone solution:** Accurately weighed quantity of 50 mg of Lawsone was transferred into 50 ml volumetric flask then diluted up to mark with methanol to obtain final concentration 0.1% w/v Lawsone solution.

❖ **Preparation of working standard solution of Diclofenac:** Accurately weighed quantity of 10 mg of Diclofenac was transferred into 10 ml volumetric flask then diluted up to mark with distilled water to obtain final concentration 1000 µg/ml of Diclofenac.

❖ **Preparation of calibration curve for Diclofenac:** In a series of 5 different 10 ml volumetric flasks 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml and 1 ml working standard solution of Diclofenac were transferred. 2 ml 0.1% Lawsone solution was added in each flasks. Volumes were made up with methanol. Spectrum of prepared standard solutions having concentration

20, 40, 60, 80 and 100 µg/ml have been recorded between 400 to 800 nm using UV-Visible spectrophotometer taking reagent blank at λmax 453 nm. Absorbance was plotted against concentration and the regression equation was determined.

5.5 Glycine

❖ **Preparation of 0.1% w/v Lawsone solution:** Accurately weighed quantity of 50 mg of Lawsone was transferred into 50 ml volumetric flask then diluted up to mark with methanol to obtain final concentration 0.1% w/v Lawsone solution.

❖ **Preparation of working standard solution of Glycine:** Accurately weighed quantity of 10 mg of Glycine was transferred into 10 ml volumetric flask then diluted up to mark with distilled water to obtain final concentration 1000 µg/ml of Glycine.

❖ **Preparation of calibration curve for Glycine:** In a series of 5 different 10 ml volumetric flasks 0.1 ml, 0.3 ml, 0.5 ml, 0.7 ml and 0.9 ml working standard solution of Glycine were transferred. 1 ml 0.1% Lawsone solution was added in each flasks. Volumes were made up with methanol. Spectrum of prepared standard solutions having concentration 10, 30, 50, 70 and 90 µg/ml have been measured between 400 to 800 nm using UV-Visible spectrophotometer taking reagent blank at λmax 455 nm. Absorbance was plotted against concentration and the regression equation was determined.

5.6 Dextrose

❖ **Preparation of 0.1% w/v Lawsone solution:** Accurately weighed quantity of 50 mg of Lawsone was transferred into 50 ml volumetric flask then diluted up to mark with ethanol to obtain final concentration 0.1% w/v Lawsone solution.

❖ **Preparation of p-nitro aniline solution:** Accurately weighed quantity of 100 mg of p-nitro aniline was transferred into 10 ml volumetric flask then diluted up to mark with ethanol to obtain 1% w/v p-Nitro aniline solution.

❖ **Preparation of working standard solution of Dextrose:** Accurately weighed quantity of 10 mg of Dextrose was transferred into 10 ml volumetric flask then diluted up to mark with distilled water to obtain final concentration 1000 µg/ml of Dextrose.

❖ **Preparation of calibration curve for Dextrose:** In a series of 5 different 10 ml volumetric flasks 0.3 ml, 0.4 ml, 0.5 ml, 0.6 ml and 0.7 ml working standard solution of

Dextrose were transferred. 1 ml 0.1% Lawsone solution and 0.6 ml p-Nitro aniline solution was added in each flasks. Volumes were made up with ethanol. Spectrum of prepared standard solutions having concentration 30, 40, 50, 60 and 70 µg/ml have been measured between 400 to 800 nm using UV-Visible spectrophotometer taking reagent blank at λmax 435 nm. Absorbance was plotted against concentration and the regression equation was determined.

5.7 L-Glutathione reduced

❖ **Preparation of 0.1% w/v Lawsone solution:** Accurately weighed quantity of 50 mg of Lawsone was transferred into 50 ml volumetric flask then diluted up to mark with methanol to obtain final concentration 0.1% w/v Lawsone solution.

❖ **Preparation of working standard solution of L-Glutathione reduced:** Accurately weighed quantity of 50 mg of L-Glutathione reduced was transferred into 50 ml volumetric flask then diluted up to mark with methanol to obtain final concentration 5000 µg/ml of L-Glutathione reduced.

❖ **Preparation of calibration curve for L-Glutathione reduced:** In a series of 6 different 10 ml volumetric flasks 0.5 ml, 1 ml, 1.5 ml, 2 ml, 2.5 ml and 3 ml working standard solution of L-Glutathione reduced were transferred. 1 ml 0.1% Lawsone solution was added in each flasks. Volumes were made up with methanol. Spectrum of prepared standard solutions having concentration 50, 100, 150, 200, 250 and 300 µg/ml have been measured between 400 to 800 nm using UV-Visible spectrophotometer taking reagent blank at λmax 455 nm. Absorbance was plotted against concentration and the regression equation was determined.

5.8 Metformin

❖ **Preparation of 0.1% w/v Lawsone solution:** Accurately weighed quantity of 50 mg of Lawsone was transferred into 50 ml volumetric flask then diluted up to mark with methanol to obtain final concentration 0.1% w/v Lawsone solution.

❖ **Preparation of working standard solution of Metformin:** Accurately weighed quantity of 10 mg of Metformin was transferred into 10 ml volumetric flask then diluted up to mark with methanol to obtain final concentration 1000 µg/ml of Metformin.

❖ **Preparation of calibration curve for Metformin:** In a series of 6 different 10 ml volumetric flasks 0.8 ml, 1 ml, 1.2 ml, 1.4 ml, 1.6 ml and 1.8 ml working standard solution of

Metformin were transferred. 2 ml 0.1% Lawsone solution was added in each flasks. Volumes were made up with methanol. Spectrum of prepared standard solutions having concentration 80, 100, 120, 140, 160 and 180 µg/ml have been measured between 400 to 800 nm using UV-Visible spectrophotometer taking reagent blank at λmax 454 nm. Absorbance was plotted against concentration and the regression equation was determined.

Method validation: Validation of developed method was carried out according to ICH guideline for Validation of Analytical Procedures Q2 (R1).

❖ **Linearity study:** Solutions having respective concentration for all were prepared from working standard solution. Prepared solutions were analyzed as per the proposed method. Five replicate analyses were carried out. The mean absorbance with its standard deviation and % relative standard deviation were calculated. Mean absorbance against concentration were plotted to obtain the calibration curves. Regression equation, co- relation coefficient were computed from calibration curve.

6. RESULTS AND DISCUSSION

6.1 Sulphanilamide (primary aromatic amine)[5]

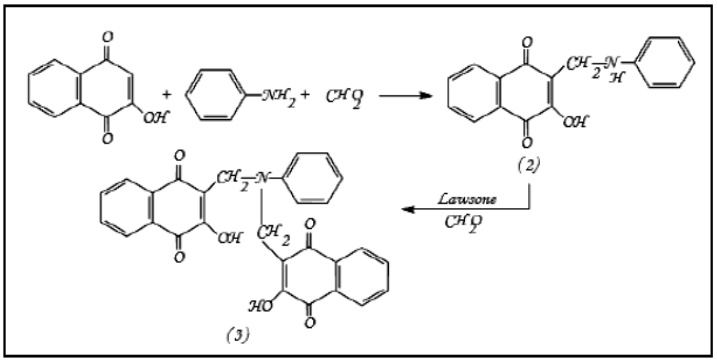


Fig 2 Reaction of lawsone with primary aromatic amine

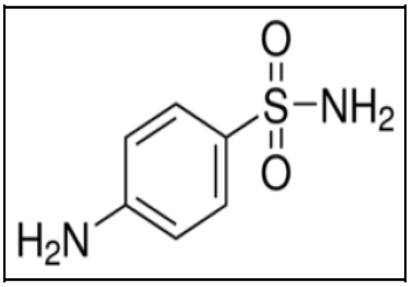


Fig 3 Structure of Sulphanilamide

Reaction Mechanism for Sulphanilamide: Primary aromatic amine containing compounds like sulphanilamide undergoes formation of mannich bases where Lawsone reacts with primary aromatic group to give to the Mannich base, which would act as a secondary amine and react further with Lawsone to give coloured compound.

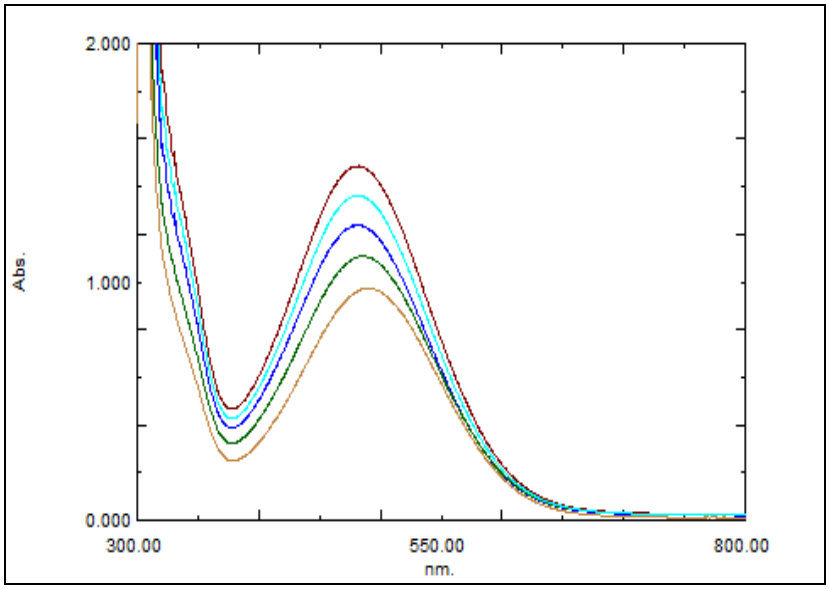


Fig. 4: Overlaid spectra of sulphanilamide (20-60 µg/ml) at λmax 482 nm.

Table. 1: Linearity Data of Sulphanilamide at 482 nm.

Sr. No.	Concentration (gm/100ml)	Absorbance*± SD	%RSD
1	0.002	0.8000±0.0039	0.489898
2	0.003	0.9620±0.0007	0.077789
3	0.004	1.1200±0.0004	0.043741
4	0.005	1.3010±0.0004	0.037655
5	0.006	1.5000±0.0048	0.326599

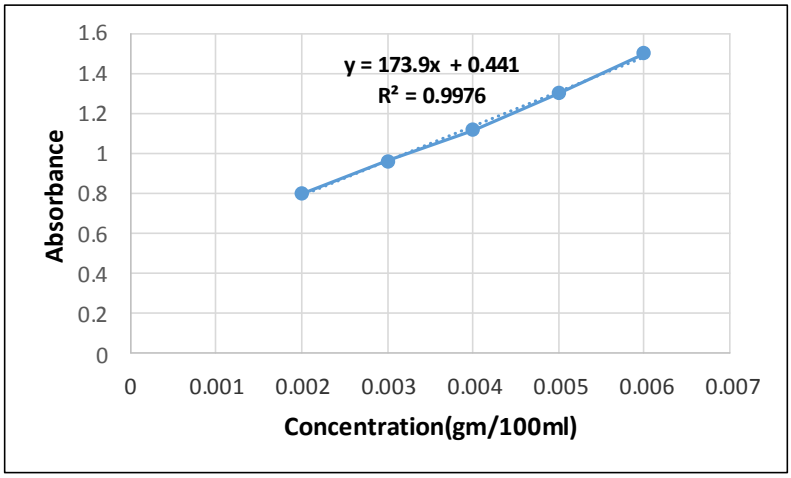


Fig. 5: Calibration curve of sulphanilamide (20-60 µg/ml) at λmax 482 nm.

Table. 2: Result of Calibration curve of sulphanilamide.

Parameter	Sulphanilamide
Regression equation	y = 173.9x + 0.441
Correlation coefficient	R^2 = 0.9976

6.2 Ramipril (secondary aliphatic amine).[5]

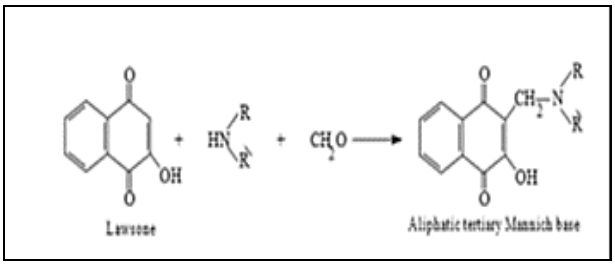


Fig. 6. Reaction of lawsone with secondary aromatic amine.

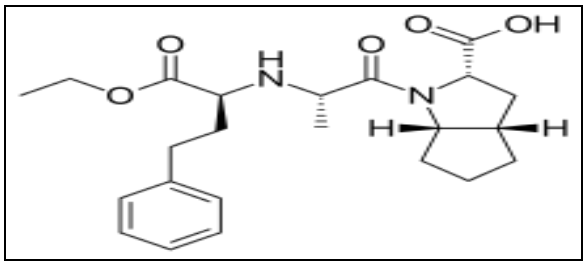


Fig. 7. Structure of Ramipril.

❖ **Reaction Mechanism for Ramipril:** Secondary aromatic amine containing compounds like ramipril undergoes formation of mannich bases where Lawsone reacts with secondary aromatic group to give aliphatic tertiary Mannich bases obtained by the reaction of secondary amines with Lawsone to give coloured compound.

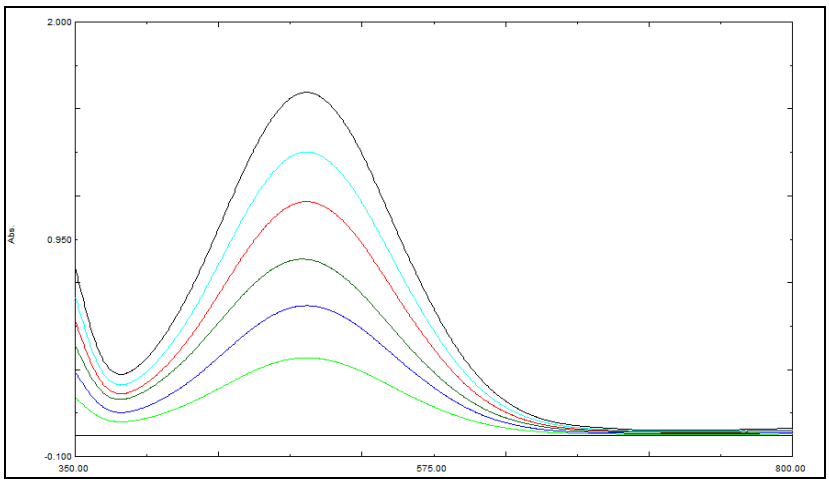


Fig. 8: Overlaid spectra of ramipril (20-70 µg/ml) at λmax 496 nm.

Table. 3: Linearity Data of Ramipril at 496 nm.

Sr. No.	Concentration (gm/100ml)	Absorbance*± SD	%RSD
1	0.002	0.3770±0.0004	0.129946
2	0.003	0.6290±0.0007	0.125747
3	0.004	0.8740±0.0007	0.085621
4	0.005	1.1320±0.0004	0.043198
5	0.006	1.3720±0.0004	0.035707
6	0.007	1.6600±0.0008	0.048193

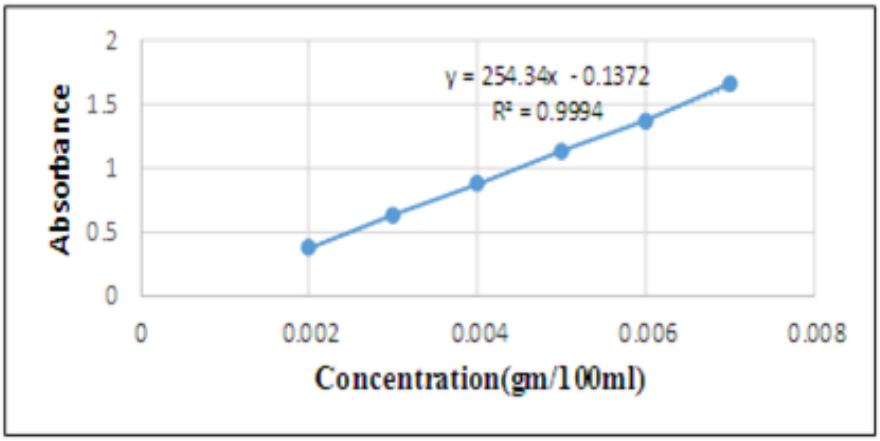
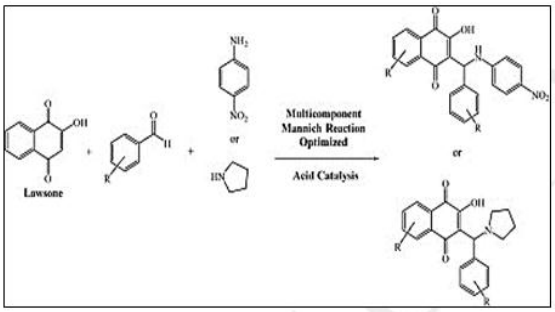


Fig. 9: Calibration curve of Ramipril (20-70 µg/ml) at λmax 496 nm.

Table. 4: Result of Calibration curve of Ramipril.

Parameter	Ramipril
Regression equation	y = 254.34x - 0.1372
Correlation coefficient	R^2 = 0.9974

6.3 Vanillin (aromatic aldehyde).[5]



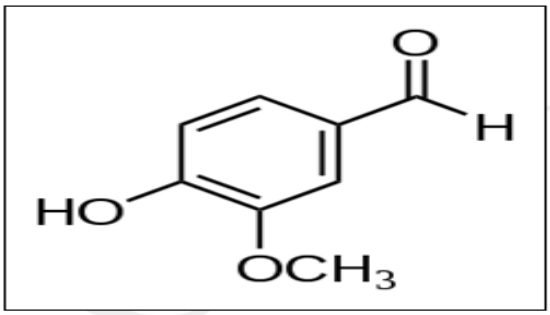


Fig. 10: Reaction of lawsone with aldehyde group. **Fig 11 Structure of Vanillin.**

❖ **Reaction Mechanism for Vanillin:** Lawsone in presence of aldehyde and p-nitro aniline undergoes mannich addition reaction which is acid catalysed to produce a coloured compound. As a result of the reaction an aminomethyl group generally replaces the active hydrogen atom to give products known as Mannich bases.

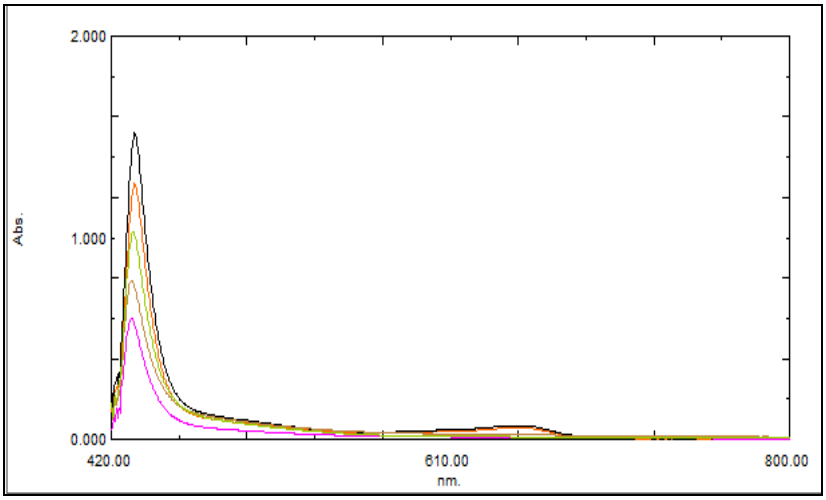


Fig. 12: Overlaid spectra of vanillin (20-60 µg/ml) at λmax 433 nm.

Table. 5: Linearity Data of Vanillin at 433 nm.

Sr. No.	Concentration (gm/100ml)	Absorbance*± SD	%RSD
1	0.002	0.5500±0.0004	0.089072
2	0.003	0.7880±0.0004	0.06217
3	0.004	1.0290±0.0004	0.047609
4	0.005	1.2680±0.0004	0.038635
5	0.006	1.5210±0.0006	0.041582

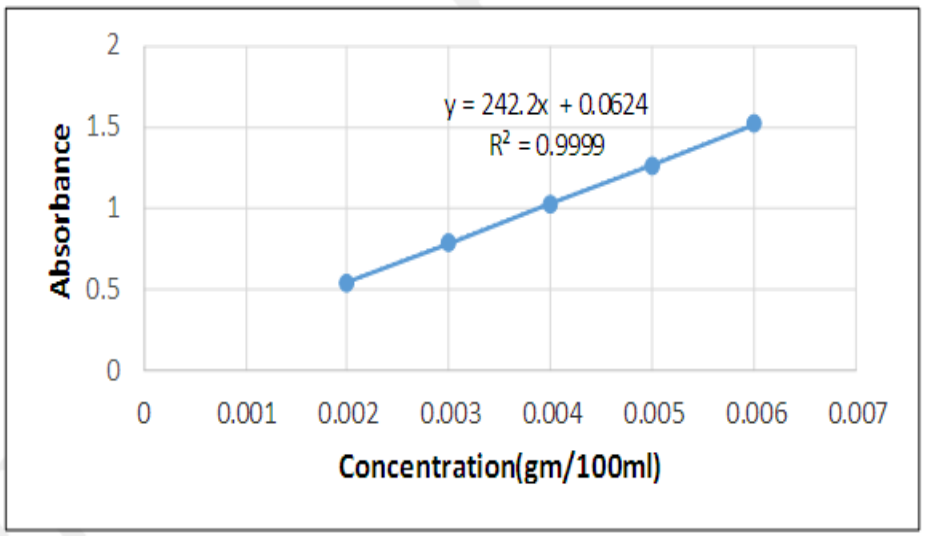
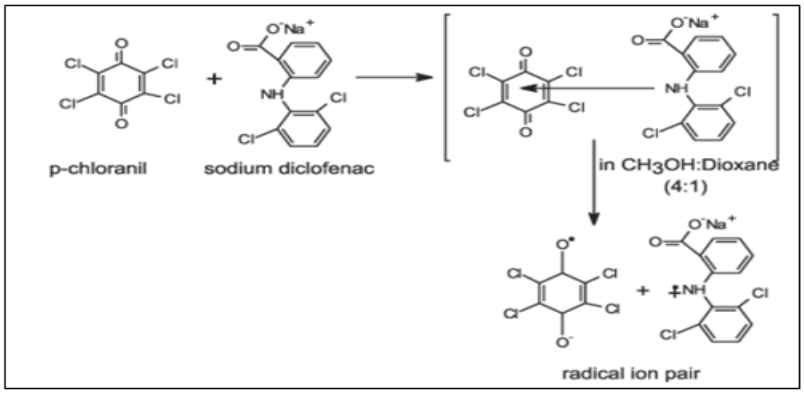


Fig. 13: Calibration curve of Vanillin (20-60 µg/ml) at λmax 433 nm.

Table. 6: Result of Calibration curve of Vanillin.

Parameter	Vanillin
Regression equation	y = 242.2x + 0.0624
Correlation coefficient	R^2 = 0.9999

6.4 Diclofenac (secondary aromatic amine).[6]



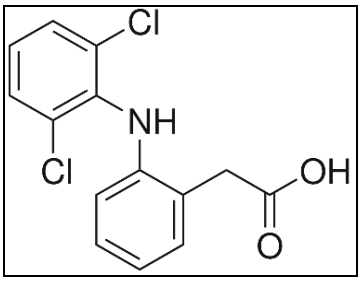


Fig. 14: Reaction of p-chloranil with diclofenac. Fig. 15: Structure of Diclofenac.

❖ **Reaction Mechanism for Diclofenac:** Lawsone undergoes the charge transfer complexation with diclofenac where diclofenac donates electron and lawsone accepts the electron.

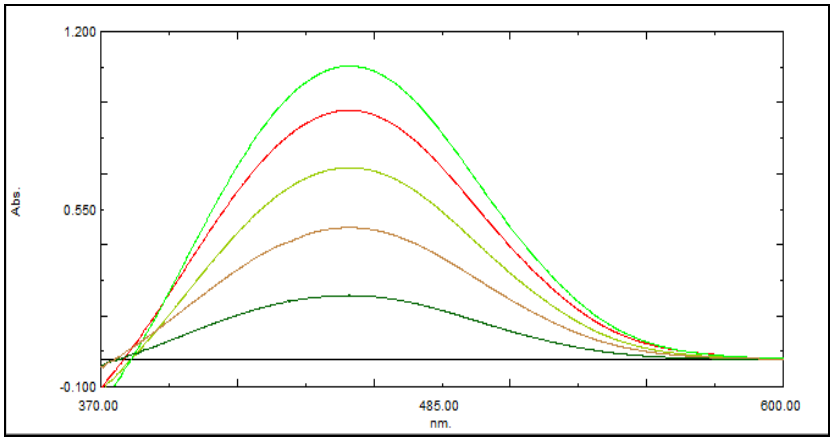


Fig. 16: Overlaid spectra of Diclofenac (20-100 µg/ml) at λmax 453 nm.

Table. 7: Linearity Data of Diclofenac at 453 nm.

Sr. No.	Concentration (gm/100ml)	Absorbance*± SD	%RSD
1	0.002	0.1800±0.0008	0.444444
2	0.004	0.3800±0.0063	1.664357
3	0.006	1.5800±0.0004	0.089072
4	0.008	1.7400±0.0004	0.662024
5	0.010	1.9400±0.0004	0.521168

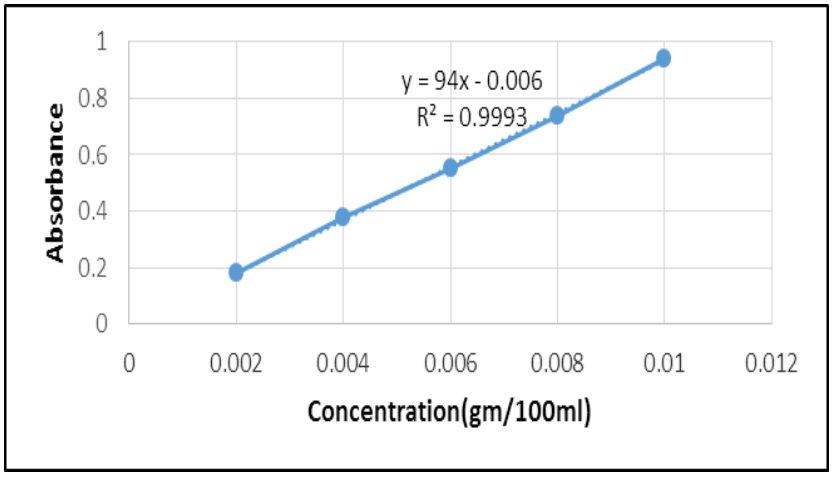
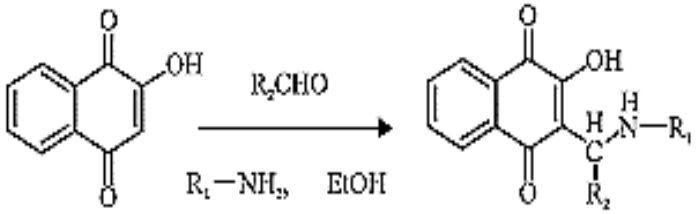


Fig. 17: Calibration curve of Diclofenac (20-100 µg/ml) at λmax 453 nm.

Table. 8: Result of Calibration curve of Diclofenac.

Parameter	Diclofenac
Regression equation	y = 94x - 0.006
Correlation coefficient	R^2 = 0.9993

6.5 Glycine (amino acid/protein).[7]



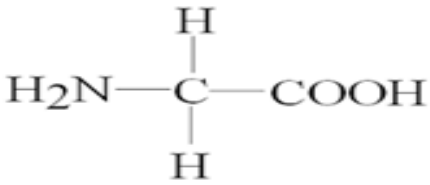


Fig. 18: Reaction of 1, 4 hydroquinone with protein (amino acid).

Fig. 19: Structure of Glycine.

❖ Reaction Mechanism for Glycine

1,4-Hydroquinones undergoes complex formation with proteins and amino acids with N-H bridge linkage to form the coloured complex.

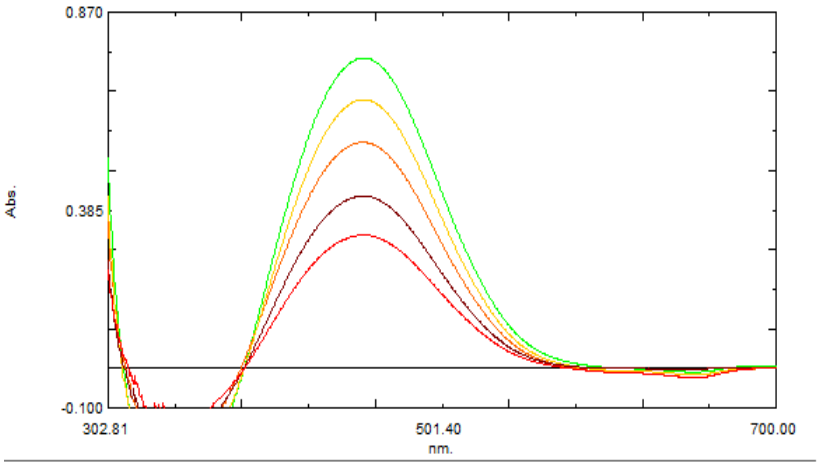


Fig. 20: Overlaid spectra of Glycine (10-90 µg/ml) at λmax 455 nm.

Table. 9: Linearity Data of Glycine at 455 nm.

Sr. No.	Concentration (gm/100ml)	Absorbance*± SD	%RSD
1	0.001	0.3240±0.0004	0.151203
2	0.003	0.4200±0.0048	1.166424
3	0.005	0.5410±0.0007	0.138324
4	0.007	0.6560±0.0009	0.149359
5	0.009	0.7810±0.0004	0.062727

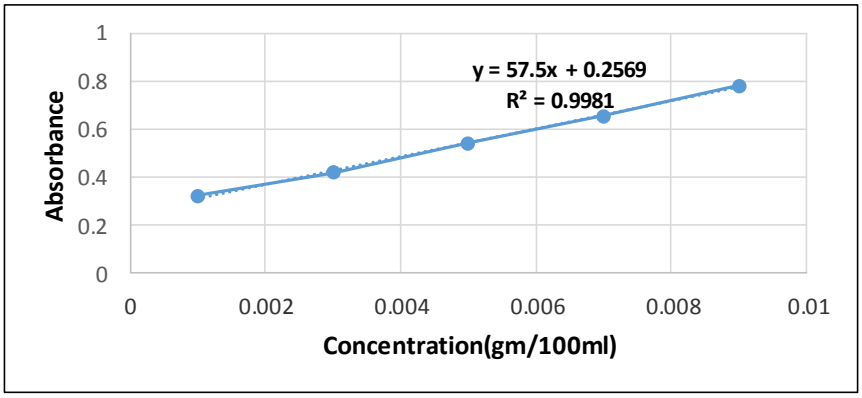
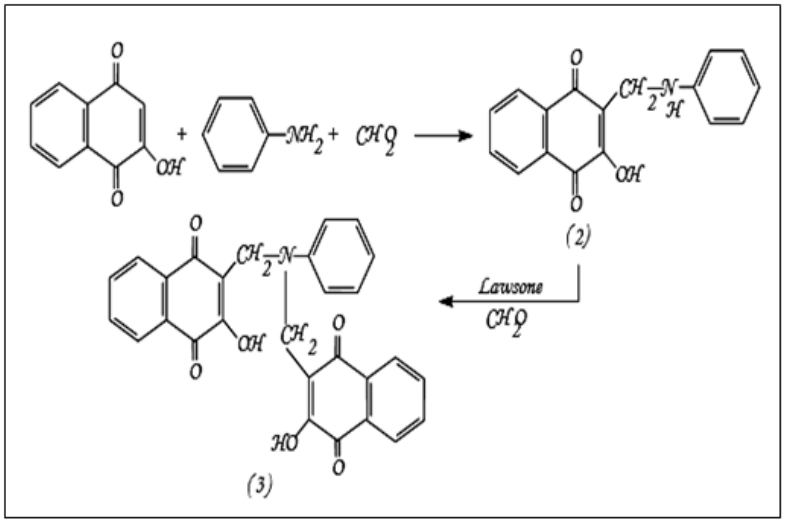


Fig. 21: Calibration curve of Glycine (10-90 µg/ml) at λmax 455 nm.

Table. 10: Result of Calibration curve of Glycine.

Parameter	Glycine
Regression equation	y = 57.5x + 0.2569
Correlation coefficient	R^2 = 0.9981

6.6 Dextrose (aliphatic aldehyde)[5]



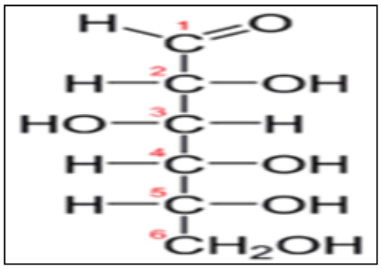


Fig. 22: Reaction of lawsone with aldehyde group. Fig. 23: Structure of Dextrose.

❖ **Reaction Mechanism for Dextrose**

Lawsone in presence of aldehyde and p-nitro aniline undergoes mannich addition reaction which is acid catalysed to produce a coloured compound. As a result of the reaction an aminomethyl group generally replaces the active hydrogen atom to give products known as Mannich bases.

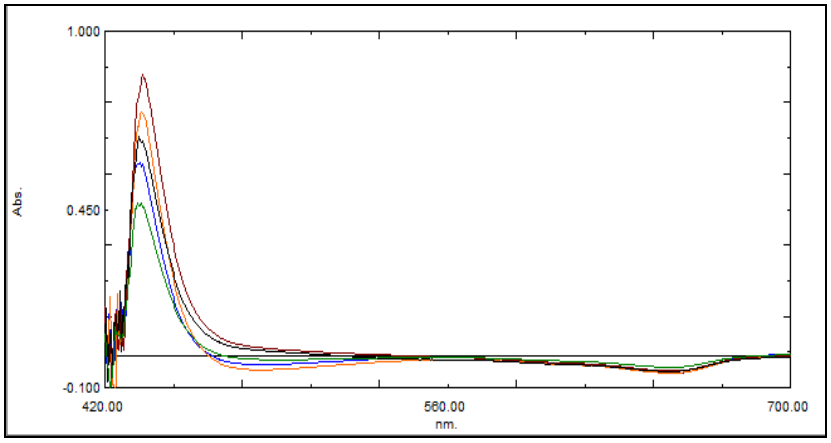


Fig. 24: Overlaid spectra of Dextrose (30-70 µg/ml) at λmax 435 nm.

Table. 11: Linearity Data of Dextrose at 435 nm.

Sr. No.	Concentration (gm/100ml)	Absorbance*± SD	%RSD
1	0.003	0.5100±0.0004	0.096058
2	0.004	0.5930±0.0004	0.082613
3	0.005	0.6700±0.0004	0.111691
4	0.006	0.7530±0.0004	0.065059
5	0.007	0.8500±0.0074	0.057635

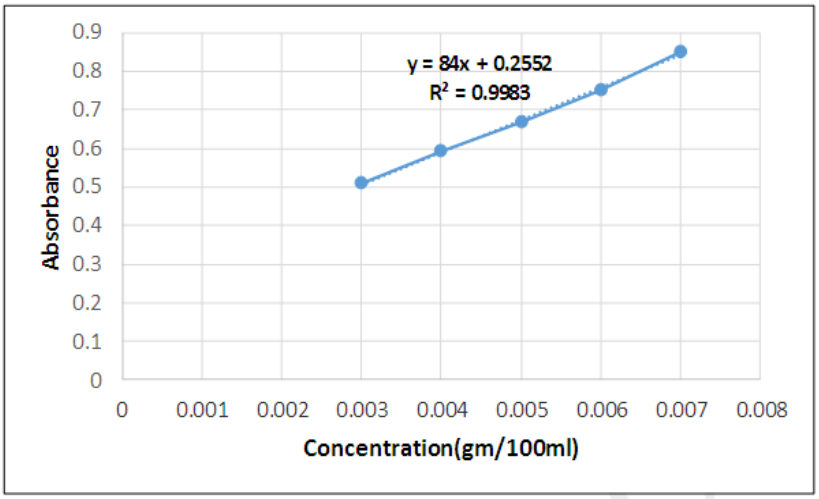


Fig. 25: Calibration curve of Dextrose (30-70 µg/ml) at λmax 435 nm.

Table. 12: Result of Calibration curve of Dextrose.

Parameter	Dextrose
Regression equation	y = 84x + 0.2552
Correlation coefficient	R^2 = 0.9983

6.7 L-Glutathione reduced (peptide)[8]

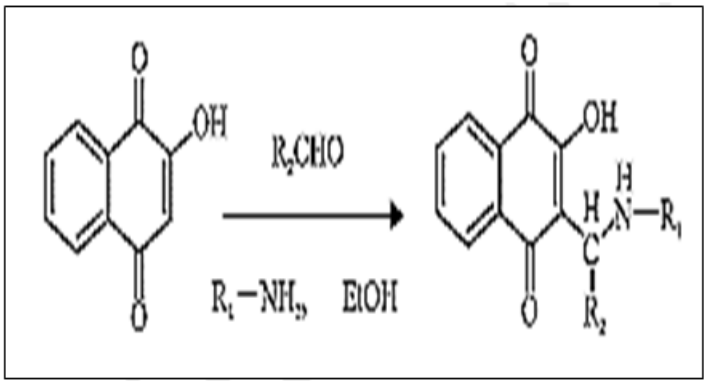


Fig. 26. Reaction of lawsone with amine group.

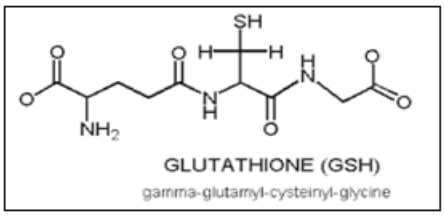


Fig. 27: Structure of L-Glutathione reduced.

❖ **Reaction Mechanism For L-Glutathione Reduced**

Lawsone for any peptide or aldehyde undergoes mannich addition reaction which is acid catalysed to produce a coloured compound. As a result of the reaction an aminomethyl group generally replaces the active hydrogen atom to give products known as Mannich bases.

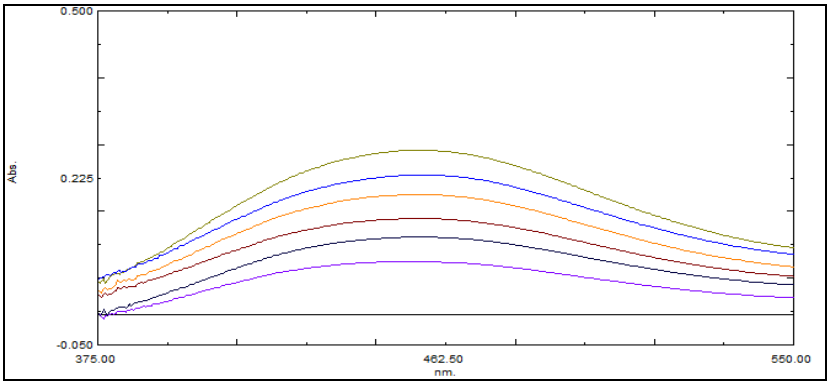


Fig. 28: Overlaid spectra of L-Glutathione reduced (50-300 µg/ml) at λmax 455 nm.

Table. 13: Linearity Data of L-Glutathione reduced at 455 nm.

Sr. No.	Concentration (gm/100ml)	Absorbance*± SD	%RSD
1	0.005	0.0870±0.0009	1.126202
2	0.010	0.1200±0.0010	0.912871
3	0.015	0.1580±0.0004	0.300616
4	0.020	0.1940±0.0003	0.20202
5	0.025	0.2300±0.0008	0.347826
6	0.030	0.2650±0.0004	0.184867

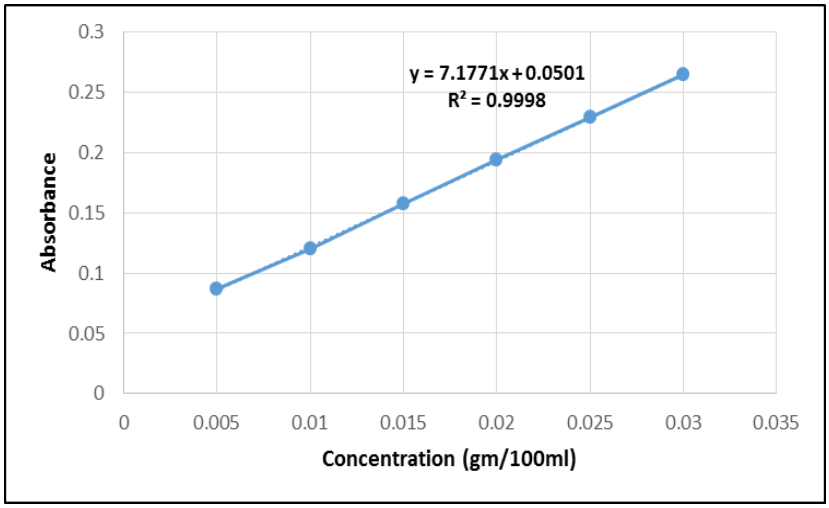


Fig. 29: Calibration curve of L-Glutathione reduced (50-300 µg/ml) at λmax 455 nm.

Table. 14: Result of Calibration curve of L-Glutathione reduced.

Parameter	L-Glutathione reduced
Regression equation	y = 7.1771x + 0.0501
Correlation coefficient	R^2 = 0.9998

6.8 Metformin (Biguanide)[8]

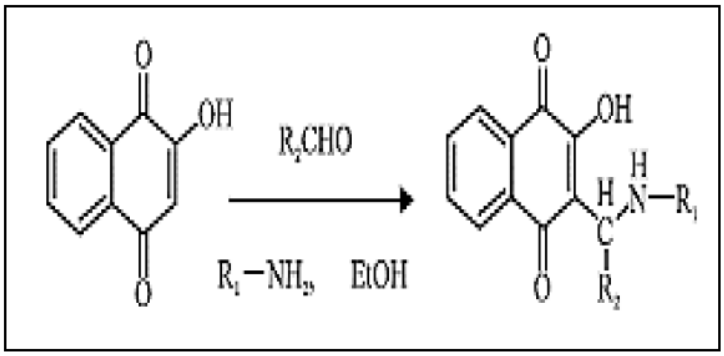


Fig 30 Reaction of lawsone with amine group

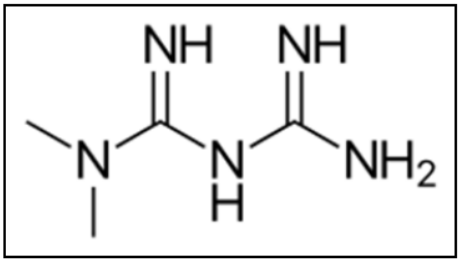


Fig 31 Structure of Metformin

❖ Reaction Mechanism Metformin

Lawsone for any biguanide or aldehyde undergoes mannich addition reaction which is acid catalysed to produce a coloured compound. As a result of the reaction an aminomethyl group generally replaces the active hydrogen atom to give products known as Mannich bases.

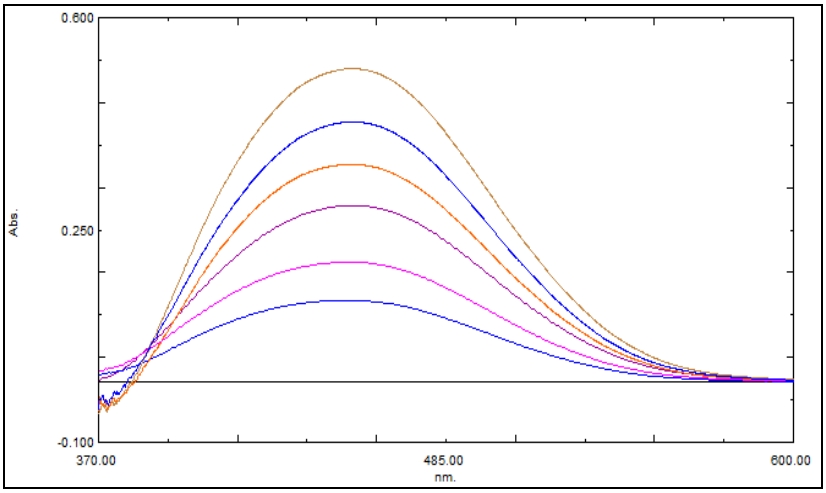


Fig. 32: Overlaid spectra of Metformin (80-180 µg/ml) at λmax 454 nm.

Table. 15: Linearity Data of Metformin at 454 nm.

Sr. No.	Concentration (gm/100ml)	Absorbance*± SD	%RSD
1	0.008	0.1340±0.0007	0.558456
2	0.010	0.2010±0.0008	0.39801
3	0.012	0.2823±0.0007	0.265285
4	0.014	0.3580±0.0004	0.136843
5	0.016	0.4280±0.0004	0.096221
6	0.018	0.5160±0.0004	0.086322

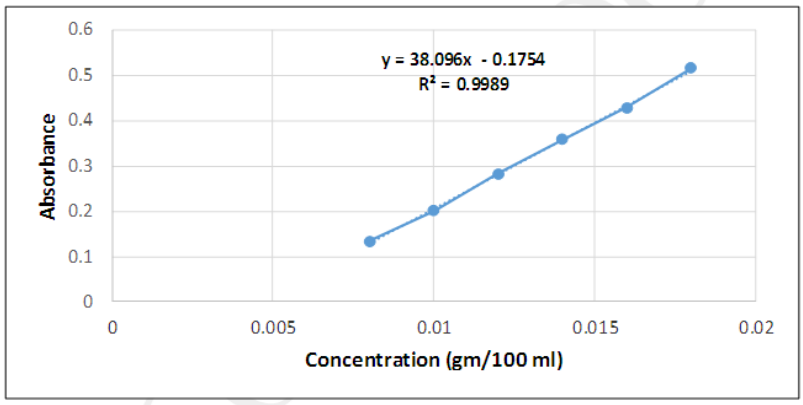


Fig. 33: Calibration curve of Metformin (80-180 µg/ml) at λmax 454 nm.

Table. 16: Result of Calibration curve of Metformin.

Parameter	Metformin
Regression equation	y = 38.096x - 0.1754
Correlation coefficient	R^2 = 0.9989

7. CONCLUSION AND ACKNOWLEDGEMENT

As per completion of the entire research, I came to clear and precise result for the estimation of various API having different functional groups. Hence this is one of the novel methods for the estimation of various drugs. I would like to express my deepest gratitude to my esteemed Guide Dr. Parula B. Patel (Principal of S. J. Thakkar Pharmacy College, Rajkot), for providing me with unfailing support, excellence guidance and continuous encouragement throughout my years of study and through the process of researching and writing this.

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