# ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF METHYLCOBALAMIN BY UFLC IN ORAL SOLID DOSAGE FORMS

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**Abstract**:
Methylcobalamin Tablets are available in the market. But the literature review reveals that there is no official monograph for Methylcobalamin Tablets in Indian Pharmacopoeia and also no analytical method reported for the analysis of Methylcobalamin by UFLC in oral solid dosage form. Spectrophotometer and RP-HPLC are the reported analytical methods for compounds either individually or in combination with other dosage form. Hence, it was felt that, there is a need of new analytical method development for the estimation of Methylcobalamin by UFLC in oral solid dosageform. Present work is aimed to develop a new, simple, fast, rapid, accurate, efficient and reproducible Liquid Chromatography method for the analysis Methylcobalamin by UFLC in solid dosage form. The developed method will be validated according to ICH guidelines. Retention time of Methylcobalamin was found to be 1.193min. and the regression equation of Methylcobalamin is y=0.0000x-0.1035 &r²=0.9999. The Method precision is 0.73%, Intermediate precision for day 01 is 052% and the intermediate precision for Day 02 is 0.55% The assay of Methylcobalamin found as 100.21%.Since the Retention time of Methylcobalamin is less than 2.0 minutes, the developed method is cost effective with short analytical time. So the method developed was simple and economical that can be adopted in regular Quality control test inIndustries.

**Key words:** Methylcobalamin, Oral solid dosage forms ,RP-HPLC, UFLC

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**Introduction**

Methylcobalamin Tablets are available in the market. But the literature review reveals that there is no official monograph for Methylcobalamin Tablets in Indian Pharmacopoeia and also no analytical method reported for the analysis of Methylcobalaminby UFLC in oral solid dosage form.

Spectrophotometer and RP-HPLC are the reported analytical methods for compounds either individually or in combination with other dosage form. Hence, it was felt that, there is a need of new analytical method development for the estimation of Methylcobalaminby UFLC in oral solid dosageform.

Present work is aimed to develop a new, simple, fast, rapid, accurate, efficient and reproducible Liquid Chromatography method for the analysis Methylcobalaminby UFLC in solid dosage form. The developed method will be validated according to ICH guidelines.

**Materials and Methods:**

**Materials**: Methylcobalamin tablet prepared in house, HPLCwater, ortho phosphoric acid, Acetonitrile, Ammonia. All the above chemicals and solvents are from Rankem.

**Instruments**:

- ElectronicsBalance-Mettler toledo
- pHmeter –Hanna, 5 Pointcalibration
- Ultrasonicator-BVKenterprises
- LC-20AD PROMINENCEUFLC
- Software: LC SOLUTIONSHIMADZU

**Methods**:

Chromatographic condition:

1. Column: INERTSIL ODS, C18 (50 mm X 3.6 mm,3µm)
2. Wavelength:352nm
3. Flowrate:0.7ml/min
4. Injection volume:2µl
5. Mobile phase: Mixture of Buffer: Acetonitrile(600:400)

**Buffer preparation**: 3.4ml of orthophosphoric acid in 1000 ml of water PH to 2.7 with ammonia.

**Diluent**: Based up on the solubility of the drugs, diluent was selected, Mobile phaseused as diluent.

**Preparation of Standard solution**: Weigh accurately and transfer about 10 mg of Methylcobalaminstandard into 100 ml volumetric standard flask. Dissolveand makeup to the volume with Mobilephase.

**Preparation of Sample solution**: Weigh accurately equivalent to 10mg of sample into 100 ml volumetric standard flask. Dissolveand makeup to the volume with Mobilephase.

**Validation**:

**System Suitability Parameters**: The system suitability parameters were determined by injecting standard solution of Methylcobalamin five times and the percentage RSD for Area and Retention Times to be checked. The % RSD for the area of five replicates of standard injections should not be more than 2.0.

**Specificity**: Checking of the interference of diluent and placebo at the retention time of Methylcobalamin in the optimized method. We should not find any interfering peaks in blank and placebo at retention times of Methylcobalamin.

**Precision**:

**Preparation of standard solution**: Weigh accurately and transfer about 10 mg of Methylcobalaminstandard into 100 ml volumetric standard flask. Dissolve and makeup to the volume with Mobilephase.

**Preparation of sample solution**: Weigh accurately equivalent to 10mg of sample into 100 ml volumetric standard flask. Dissolve and makeup to the volume with Mobilephase.

**Linearity (forMethylcobalamin):** Preparation of standard stock solution: Weighed accurately and transferred about 100mg of Methylcobalamin standard into 200 ml volumetric standardflask. 50%MethylcobalaminStandard solution: 5ml standard stock solutions was pipetted out and made up to 50ml.

75% MethylcobalaminStandard solution: 7.5ml standard stock solutions was pipetted out and made up to 50ml.

100% MethylcobalaminStandard solution: 10ml standard stock solutions was pipetted out and made up to 50ml.

125% MethylcobalaminStandard solution: 12.5mlstandard stock solutions was pipetted out and made up to50ml.

150% MethylcobalaminStandard solution: 15mlstandard stock solutions was pipetted out and made up to50ml.

**Accuracy**:

Preparation of Standard stock solution: Weigh accurately and transfer about 10 mg ofMethylcobalamin standard into 100 ml volumetric standard flask. Dissolveand makeup to the volume with Mobilephase.

For preparation of 80% solution: Weigh accurately and transfer powdered sample equivalent to about 8 mg of Methylcobalamin into 100 ml volumetric standard flask. Dissolve and

makeup to the volume with Mobilephase. Samples were prepared in triplicates from a uniform mixture.
For preparation of 100% solution: Weigh accurately and transfer powdered sample equivalent to about 10 mg of Methylcobalamin into 100 ml volumetric standard flask. Dissolveand makeup to the volume with Mobilephase. Samples were prepared in triplicates from a uniform mixture.
For preparation of 120% solution: Weigh accurately and transfer powdered sample equivalent to about 12 mg of Methylcobalamin into 100 ml volumetric standard flask. Dissolveand makeup to the volume with Mobilephase. Samples were prepared in triplicates from a uniform mixture.

**Results and Discussion:**

Method development: Method development was done by changing various, mobile phase ratios, buffers etc.

**Mobilephase** **:** Buffer: Acetonitrile (600:400)

**Flow rate** **:**0.7ml/min

**Column** **:** INERTSIL ODS C18, 3.6mm X 50 mm;3µ

**Detectorwavelength** **:**352nm

**Injectionvolume** **:**2µL

**Runtime** **:**6min

**Diluent** **:** Mobilephase

**Results** **:** Retention time and peak shape isgood.

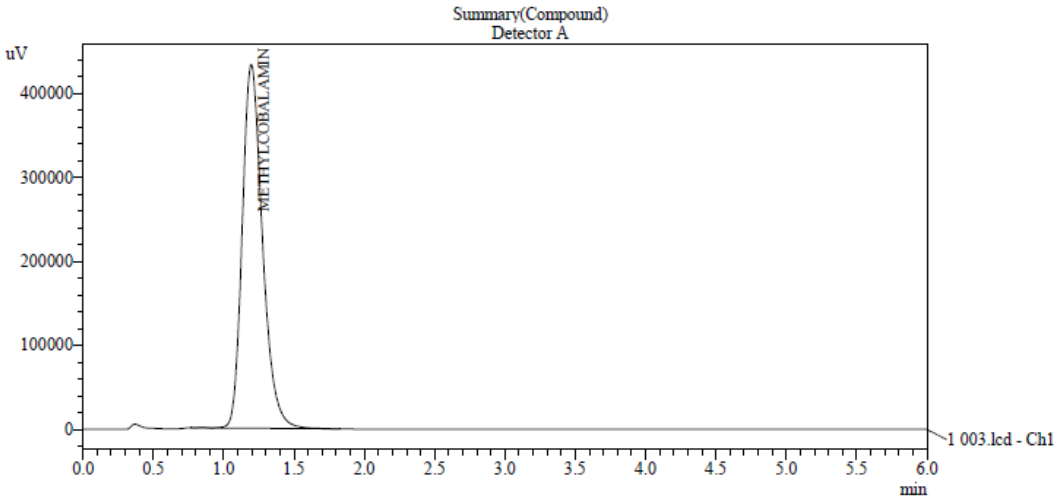


**Figure-1: Optimized Chromatogram**

**Observation:** Methylcobalamin was eluted at 1.193 min. and the system suitability met. so this method was optimized and to be validated.
**System suitability:** All the system suitability parameters were within the range and satisfactory as per ICH guidelines.

**TRable-1: System suitability parameter for Methylcobalamin**

| Sample ID | Methylcobalamin | |
|---|---|---|
| | **RT** | **AREA** |
| Injection -01 | 1.193 | 4331240 |
| Injection -02 | 1.195 | 4356897 |
| Injection -03 | 1.194 | 4357895 |
| Injection -04 | 1.191 | 4326589 |

| | | |
|---|---|---|
| Injection -05 | 1.191 | 4421543 |
| Average: | 1.193 | 4358833 |
| SD: | 0.00 | 37875 |
| % RSD: | 0.15 | 0.87 |

**Discussion**: The percentage RSD of Five replicate standard solution injection was 0.15 for Retention time and 0.87 for Area which meets the requirement of less than 2. All the system suitable parameters were passed and were within the limits.

**Specificity:**

**Table-2: Specificity for Methylcobalamin**

| Sample ID | Methylcobalamin | |
|---|---|---|
| | **RT** | **AREA** |
| BLANK | 1.193 | No peak observed |
| STANDARD | 1.193 | 4358833 |
| PLACEBO | 1.193 | No peak observed |

**Discussion**: Retention time of Methylcobalaminwas eluted at 1.193min. We did not find any interfering peaks in blank and placebo at retention times of Methylcobalamin in this method. So .this method was said to be specific.

**Linearity**:

**VALIDATION PARAMETER : LINEARITY**

**LABEL CLAIM:**

METHYLCOBALAMIN 1500 mcg — Std purity : 90.89 % — Factor : 1.0000

**METHYLCOBALAMIN linearity stock dilution:**

110.35 mg standard diluted to 100 ml (Stock Soln.)

| *Soln. ID* | *Linearity solution dilutions:* | | | | *Concentrations* | |
|---|---|---|---|---|---|---|
| *50.0% :* | 2.5 | ml | Stock soln diluted to | 50 | ml | 50.15 mcg/ml |
| *75.0% :* | 3.8 | ml | Stock soln diluted to | 50 | ml | 76.23 mcg/ml |
| *100.0% :* | 5 | ml | Stock soln diluted to | 50 | ml | 100.30 mcg/ml |
| *125.0% :* | 6.3 | ml | Stock soln diluted to | 50 | ml | 126.37 mcg/ml |
| *150.0% :* | 7.5 | ml | Stock soln diluted to | 50 | ml | 150.45 mcg/ml |

| | | | | | |
|---|---|---|---|---|---|
| Linearity Concentrations--> | 50.15 | 76.23 | 100.30 | 126.37 | 150.45 |
| Observed Area--> | 2175125 | 3285716 | 4340255 | 5436035 | 6509638 |

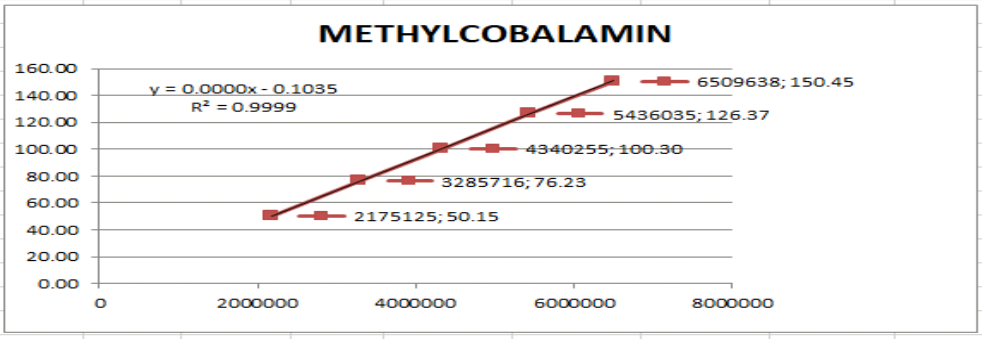


**Figure-2: Validation Parameter-Linearity**

**Discussion**: Five linear concentrations of Methylcobalaminwere injected.The area Vs concentration was plotted and the linearity equations obtained for Methylcobalaminy = 0.0000x–0.1035. Correlation coefficient obtained was$R^2$ =0.9999 for the Methylcobalamin

**Precision**: Six independent samples were prepared and analysed as per the test method .The percentage RSD for the content of Methylcobalamin was calculated for the samples and found 0.52 which is less than 2.0 and meets the requirements.

**Accuracy**: Three levels of Accuracy samples in triplicates were prepared and analysed as per the test method. The assay percentage were calculated for the each samples and found the minimum

assay is 99.82 and the maximum assay percentage is 100.94 which are well within the limit of 98.0 to 102.0.

**Robustness:**

Robustness conditions like Flow minus (0.6ml/min), Flow plus (0.8ml/min), Wavelength minus and Wavelength plus was maintained and samples were injected. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

| **Parameters** | | **Methylcobalamin** | **LIMIT** |
|---|---|---|---|
| **Linearity:** Regression equationY=mx+c) | | y = 0.0000x–0.1035 ($r^2$=0.9999) | $r^2$ not less than 0.99 |
| **Assay (% mean assay)** | | 100.21% | 98%-102% |
| **Specificity** | | Specific | No interference of any peak |
| **Method precision %RSD** | | 0.73% | RSD NMT 2.0% |
| **Intermediate precision - DAY 01 %RSD** | | 0.52% | RSD NMT 2.0% |
| **Intermediate precision - DAY 02 %RSD** | | 0.55% | RSD NMT 2.0% |
| **Accuracy %** | | 99.82% to 100.94% | 98%-102% |
| **Robustness** | **FM** | 0.33% | %RSD NMT 2.0 |
| | **FP** | 0.22% | |
| | **WM** | 0.63% | |
| | **WP** | 0.68% | |

**Conclusion**:

A simple, Accurate, precise method was developed for the simultaneous estimation of the Methylcobalaminin solid dosage form. Retention time of Methylcobalaminwas found to be 1.193min. and the regression equation of Methylcobalamin is y=0.0000x-0.1035 &$r^2$=0.9999.

The Method precision is 0.73%, Intermediate precision for day 01 is 052% and the intermediate precision for Day 02 is 0.55%. The assay of Methylcobalamin found as 100.21%.Since the Retention time of Methylcobalamin is less than 2.0 minutes, the developed method is cost effective with short analytical time. So the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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