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# Implementation of Qbd Approach to Develop and Validate HPTLC Method for simultaneous estimation of Aliskiren, Amlodipine and Hydrochlorothiazide in Pharmaceutical Formulation

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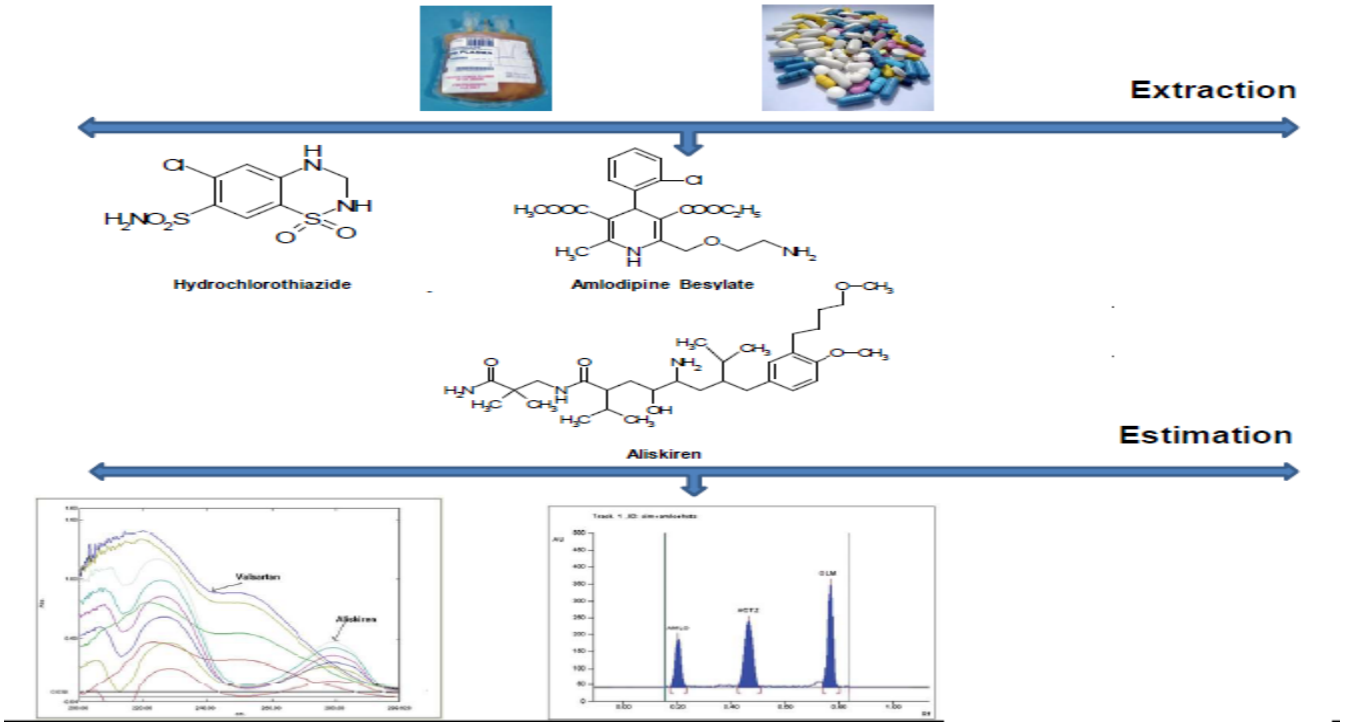
## Graphical Abstract



**Abstract:**
A High performance thin layer chromatographic method for simultaneous estimation of Aliskiren, Amlodipine besylate and Hydrochlorothiazide was developed and validated as per ICH guidelines. Moreover, robustness testing was performed applying a central composite design with k factor having 2 k factorial runs, 2k axial experiments and two center points. High performance thin layer chromatographic separation was performed on aluminium plates recoated with silica gel 60F 254 and acetonitrile: methanol: strong ammonis (10:10:0.1, v/v) as optimized mobile phase. The detection wavelength for simultaneous estimation of three drugs was 250 nm. The Rf values for Aliskiren, Amlodipine besylate and Hydrochlorthiazide were 0.54, 0.32 and 0.78 for, respectively. Percent recoveries in terms of accuracy for the marketed formulation was found to be 101.3-104.4, 100.7-104 and 101.5-103.9 for, Aliskiren, Amlodipine besylate and Hydrochlorthiazide, respectively. The pooled % relative standard deviation values for repeatability studies and intermediate precision studies was found to be less than 2% for Aliskiren, Amlodipine besylate and Hydrochlorthiazide, respectively. All these three factors (methanol content, developing distance and band size) were evaluated in the robustness testing by central composite design and these were found to have an insignificant effect on the retention factor. However, methanol content in total mobile phase as a factor appeared to have significant effect on robustness, compared to band size and developing distance and hence it is important to be carefully controlled. In summary, a novel, simple, accurate and reproducible high performance thin layer chromatographic method was developed, which would be of use in quality control of these tablets.

**Keywords:** Aliskiren, Amlodipine besylate, Hydrochlorthiazide, high performance thin layer chromatography (HPTLC) method, Validation, Central Composite Design.)

# Introduction

Recently, HPTLC is widely employed for the quantification of drugs because of low maintenance cost, lower analysis time, low mobile phase consumption per sample and need for minimum sample clean-up. It facilitates automated application of sample and scanning of plate. this research paper describes the development of HPTLC method for simultaneous estimation of Aliskiren(ALK), Amlodipine(AML) and Hydrochlorothiazide(HTZ) using Design of Experiment (DoE) approach for method validation.

DoE is based on the principle of use of experimental design, generation of mathematical equations (models) and graphical outcomes, employing various rational combination of factors. Experimental design procedures are very useful in pharmaceutical development including formulation development and analytical method optimization and validation. Experimental design methodology has proved , and are more effective than the traditional one-variable-at-a-time approach to be a useful tool for method validation, as it allows the investigation of simultaneously changing factors.

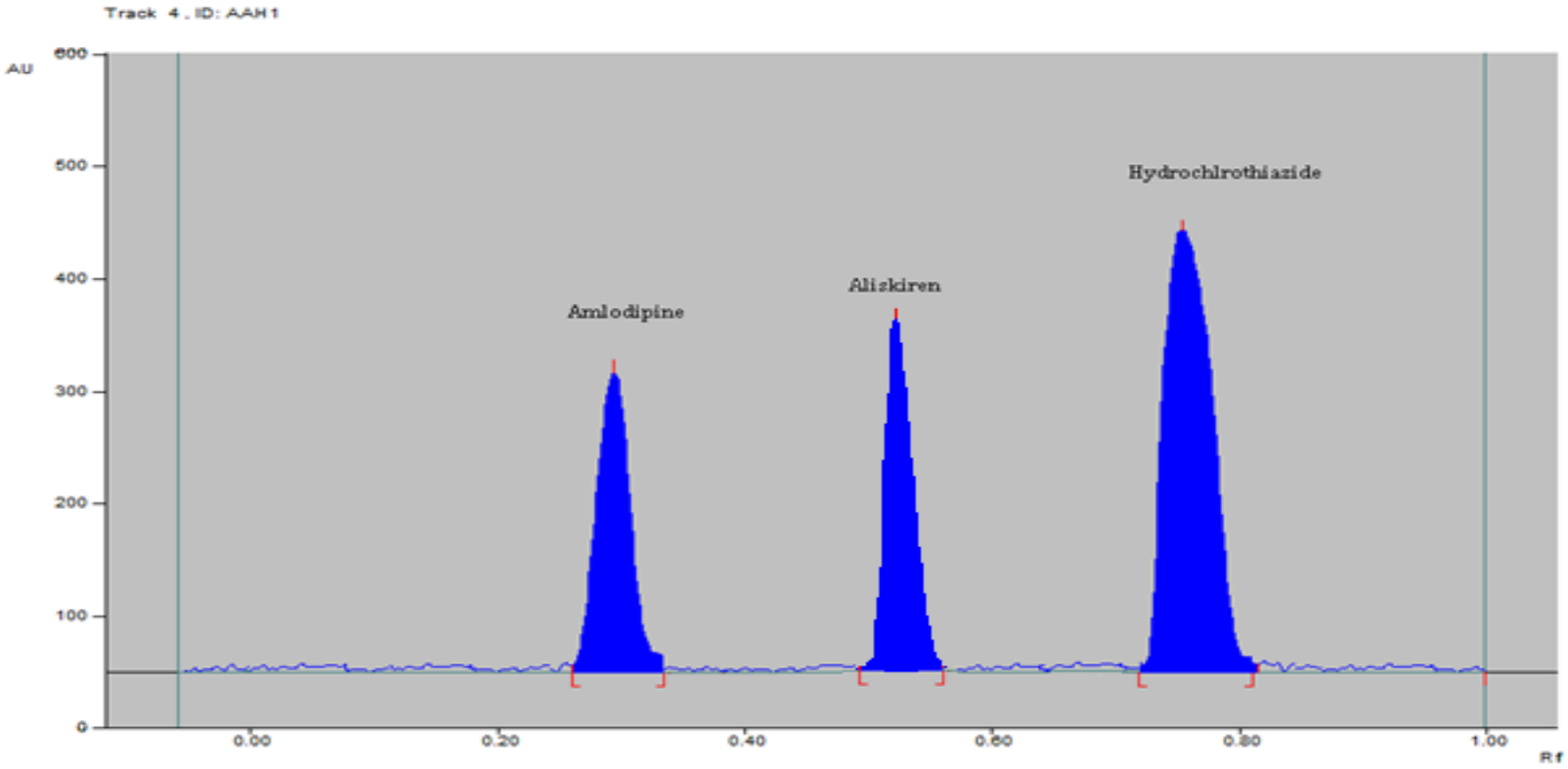
# Results and discussion

- Various combinations of solvents in different ratios like methanol, ethyl acetate, chloroform, toluene, hexane, acetonitrile, strong ammonia, glacial acetic acid and formic acid were tried for resolving the peaks of ALK, AML and HCTZ.

- Final optimized mobile phase, of **acetonitrile: methanol: strong ammonia (10:10:0.1, v/v)** which gave highest resolution and Rf values **of 0.54 ± 0.015, 0.32 ±0.007 and 0.78 ±0.011** for ALK, AML and HCTZ, respectively at detection wavelength of **250 nm**

# Results and discussion

## Chromatograms of ALK, AML and HCTZ Standard



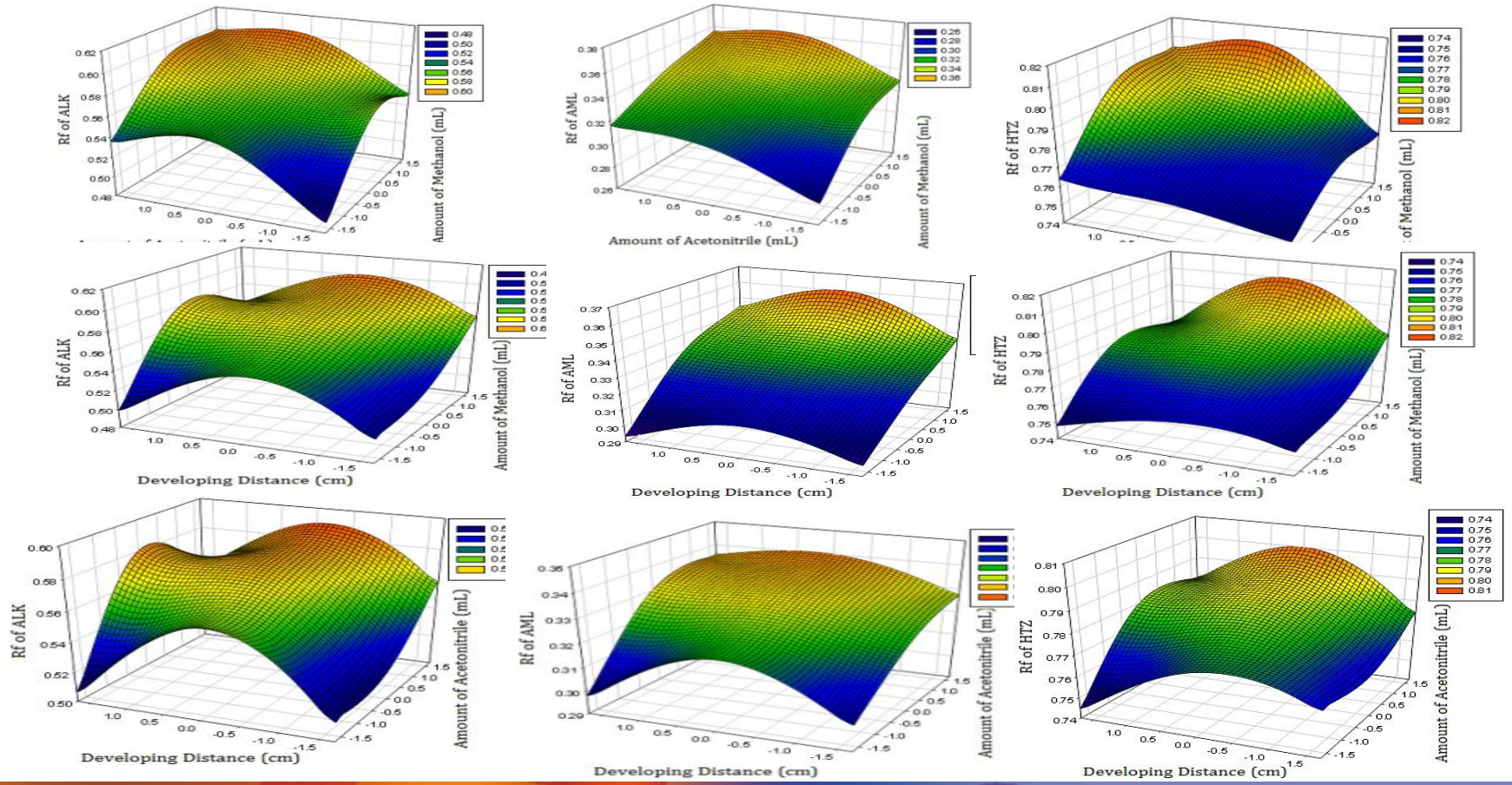
# Results and discussion

## Analytical Parameters of Proposed HPTLC method

| PARAMETERS | HPTLC Method | | |
|---|---|---|---|
| | AML | ALK | HTZ |
| Concentration range (ng/band, n=6) | 500-3000 | 1000-20000 | 500-3000 |
| Slope ± SD | 3.53414±0.0346 | 0.408±0.0063 | 4.302±0.206 |
| Intercept ± SD | 103.1867±1.98 | 209.32±36.93 | 11608±380.18 |
| Correlation coefficient($r^2$)±SD | 0.998±0.0012 | 0.996±0.0011 | 0.996±0.00089 |
| LOD | 71.84 | 321.39 | 157.05 |
| LOQ | 217.71 | 976.95 | 472.90 |
| Accuracy (% recovery, n = 6) | 100.72 ± 0.47 | 100.23 ± 1.02 | 99.63 ± 0 .64 |
| Precision (%RSD) | | | |
| Repeatability ($n$ = 3) | 0.45-1.26 | 0.27-1.63 | 0.57-1.65 |
| Intermediate precision ($n$ =3 ) | 0.69-0.81 | 0.57-1.03 | 0.94-1.79 |

# Results and discussion

Effect of amount of methanol, amount of acetonitrile and developing distance on $R_f$ of ALK(a), $R_f$ of AML (b), $R_f$ of HTZ (c) Three-dimensional plot



# Results and discussion

## Regression Analysis of Central Composite Model For Robustness Study

| Coefficients | $R_f$ of ALK | | $R_f$ of AML | | $R_f$ of HTZ | |
|---|---|---|---|---|---|---|
| | FM | RM | FM | RM | FM | RM |
| BO | 0.57219 | 0.56067 | 0.32131 | 0.326 | 0.78096 | 0.77733 |
| B1 | 0.01641 | 0.01641 | 0.014211 | 0.014211 | 0.01201 | 0.01201 |
| B2 | 0.01102 | - | 0.007588 | 0.007588 | 0.00809 | 0.00809 |
| B3 | 0.00885 | 0.00885 | 0.001964 | - | 0.00246 | - |
| B12 | 0.00628 | - | -0.00125 | - | 0.0025 | - |
| B23 | 0.00280 | - | 0.00125 | - | 0.00296 | - |
| B13 | 0.00608 | - | 0.00125 | - | 0.0025 | - |
| B11 | -0.0013 | - | 0.003484 | - | 0.00044 | - |
| B22 | -0.0013 | - | 0.001717 | - | 0.00044 | - |
| B33 | -0.0001 | - | 0.000408 | - | -0.0049 | - |
| B123 | 0.0025 | - | 0.00125 | - | 0.0025 | - |

# Results and discussion

## Assay Result of Tablet Dosage Form of Aliskiren and Amlodipine

| Sample No. | Label Claim | | | Amount Found | | | % Label Claim | | |
|---|---|---|---|---|---|---|---|---|---|
| | ALK mg/tab | AML mg/tab | HTZ mg/tab | ALK mg/tab | AML mg/tab | HTZ mg/tab | ALK mg/tab | AML mg/tab | HTZ mg/tab |
| **1** | 150 | 5 | 12.5 | 151.69 | 5.035 | 12.472 | 101.13 | 100.7 | 99.78 |
| **2** | 150 | 5 | 12.5 | 151.78 | 4.96 | 12.34 | 101.29 | 98.29 | 99.75 |
| **3** | 150 | 5 | 12.5 | 148.77 | 5.05 | 12.57 | 98.1 | 100.16 | 101.56 |
| **4** | 150 | 5 | 12.5 | 148.41 | 5.02 | 12.55 | 98.94 | 101.44 | 100.47 |
| **5** | 150 | 5 | 12.5 | 151.75 | 4.97 | 12.45 | 101.27 | 99.43 | 98.63 |
| **6** | 150 | 5 | 12.5 | 148.71 | 5.06 | 12.65 | 98.14 | 101.64 | 100.23 |
| **Mean** | | | | 149.93 | 5.01 | 150.18 | 5.01 | 12.50 | 99.81 |
| **S.D.** | | | | 1.55 | 0.04 | 1.70 | 0.04 | 0.11 | 1.03 |

## Conclusions

- The developed method was found to be novel, simple, accurate, precise, specific and reproducible for the simultaneous estimation of ALK, AML and HCTZ in bulk and tablet formulations.

- Methanol content in mobile phase appeared to have significant effect on robustness, compared to other factors and hence it was important to be carefully controlled.

- Use of experimental design reduce the number of the require experiments for the robustness study of HPTLC method

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