International Journal of
Science Inventions Today
www.ijsit.com
ISSN 2319-5436
Research Article

# DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR DETERMINATION OF THEOPHYLLINE AND 1METHYL URIC ACID FROM HUMAL PLASMS

Bharat

## ABSTRACT

A stable, simple, rapid, precise, accurate HPLC method for analysis of Theophyllinee and 1-Methyl Uric Acid was developed and validated as per ICH guidelines without need of any internal standard. Separation was carried out using X'terra RP18 (250*4.6) mm, 5µ column with potassium dihydrogen orthophosphate buffer (pH 3): acetonitrile (30:70 v/v) as mobile phase with flow rate 1 mL min-1. The parameters studied were retention time, linearity and range, accuracy, precision. The proposed method can be used for determination of Theophylline and 1-Methyl Uric Acid from Human plasma.

**Keywords:** Theophylline, HPLC, Validation.

## INTRODUCTION

In the early 20th century, the German chemist Paul Ehrlich was developing theories of selective toxicity based largely on the ability of certain dyes to kill microbes. Gerhard Domagk, who would later win a Nobel Prize for his efforts, made a major breakthrough in 1932 with the discovery of the antibacterial prontosil red. Further investigation into the active chemicals involved led to the discoveries of antibacterial sulfonamides (1935) by Daniel Bovet and his team at Pasteur Institute, then of Theophyllinee (1937) independently by Ernest Fourneau in France and Gladwin Buttl in United-Kingdom.

## MATERIAL AND METHOD

### Analytical Section:

Extraction of THEOPHYLLINE and 1-METHYL URIC ACID from plasma was achieved by a simple deproteination with trichloroacetic acid; this results in easy, rapid, and convenient separation of the analytes[16, 18]. The chromatograms obtained under the assay conditions used were clean, despite injection of the sample on to the column without pre-purification.

Ion pair chromatography with 1-hexanesulfonic acid sodium salt in the mobile phase, results in retention of THEOPHYLLINE, a polar molecule of low molecular weight, on the column by the formation of a complex. It is important the proportion of 1-hexanesulfonic acid sodium salt in the mobile phase is relatively high at the beginning of the chromatographic run (gradient starts with 90% of this phase). Under these conditions the hydrocarbon chain of the ion pair interacts with the octadecylsilane chains of the stationary phase and the complex is retained long enough to be chromatographically separated.

This HPLC method enabled rapid simultaneous measurement of THEOPHYLLINE and its acetylated metabolite 1-METHYL URIC ACID in plasma samples. Use of the gradient described resulted in sharp and symmetrical peaks. Total analysis time, including sample pretreatment and rapid elution, was less than 15 min.

### HPLC method development for Pure Theophyllinee (THEOPHYLLINE) And MonoacetylTheophylline (1-METHYL URIC ACID):

The linearity of the response of the drug was verified from 0.5 to 15 ng/ml concentrations. The calibration graphs were obtained by plotting the response versus the concentration. The calibration curve was found to be linear in the aforementioned concentrations. The correlation coefficient ($r^2$) of determination was 1 which indicates that the method is accurate.

| Sr. No. | Theophylline Concentration (µg/ml) | Area | 1-Methyl Uric Acid Concentration | Area |
|---|---|---|---|---|
| **1** | 1.043165858 | 80059.1 | 0.978702662 | 85173 |
| **2** | 2.085241811 | 161304 | 1.956382769 | 171608 |
| **3** | 3.144483784 | 244733 | 2.95016811 | 260367 |
| **4** | 4.190615444 | 323979 | 3.9316533 | 344675 |
| **5** | 5.248274957 | 406166 | 4.923953971 | 432113 |
| **6** | 6.286620077 | 487541 | 5.898133795 | 518685 |
| **7** | 7.346084223 | 569989 | 6.89212758 | 606400 |
| **8** | 8.384563486 | 652471 | 7.866433258 | 694151 |
| **9** | 9.431669774 | 734634 | 8.848832848 | 781562 |
| **10** | 10.48033756 | 812333 | 9.832697443 | 864225 |
| | Slope | 86799 | Slope | 81587 |
| | Intercept | -1497 | Intercept | -1407 |
| | Correlation co-efficient | 1 | Correlation co-efficient | 1 |

**Table 1:** Linearity of Theophyllinee and MonoacetylTheophylline for HPLC method development

| Actual Value (µgmL-1) | THEOPHYLLINE | | | 1-METHYL URIC ACID | | |
|---|---|---|---|---|---|---|
| | 0.8 | 5 | 13 | 0.8 | 5 | 13 |
| **Mean concentration found (µgmL-1)** | 0.9 | 4.5 | 12.7 | 0.7 | 4.6 | 12.5 |
| **Number of replicates** | 10 | 10 | 10 | 10 | 10 | 10 |
| **Standard deviation (SD)** | 0.04 | 0.3 | 0.6 | 0.02 | 0.06 | 0.2 |
| **CV (%)a** | 4.5 | 7.0 | 5.0 | 2.9 | 4.3 | 0.9 |
| **Accuracy (%)b** | 10.4 | -9.8 | -2.2 | -4.3 | -6.9 | -3.7 |

**Table 2:** Within- run precision and accuracy of the HPLC method

CV = (SD/Mean) x 100%

{( Amount found)-(amount added)}/(amount added)}] x 100%

## Linearity:

When average peak area was plotted against the Theophylline concentration and its metabolite in plasma the plots were linear in the range 0.5 to 15.0 μg mL−1. Typical calibration plots for plasma extracts had good correlation coefficients (0.9998 for Theophylline and 0.9905 for MonoacetylTheophylline; n = 6 calibration points).

## Limits of Quantification and Detection:

The limit of quantification, defined as the lowest concentration that could be measured with accuracy and precision, i.e. within ±20% of the actual value[20], was 0.5 μg mL−1. The lower limits of detection of THEOPHYLLINE and 1-METHYL URIC ACID (three times the baseline noise) were 0.24 and 0.12 μg mL−1, respectively.

## Intra-Day Repeatability:

Assay performance was evaluated as intra-day accuracy and precision, determined by replicate analysis of QC samples.

These results show the repeatability of the assay, including both sample processing and chromatographic measurement, is good. Small deviations from perfect accuracy were observed (i.e. 10.4% at most).

| **Actual Value (μgmL-1)** | **THEOPHYLLINE** | | | **1-METHYL URIC ACID** | | |
|---|---|---|---|---|---|---|
| | 0.8 | 5 | 13 | 0.8 | 5 | 13 |
| **Mean concentration found (μgmL-1)** | 0.84 | 5.0 | 13.1 | 0.8 | 4.7 | 12.8 |
| **Number of replicates** | 6 | 6 | 6 | 6 | 6 | 6 |
| **Standard deviation (SD)** | 0.04 | 0.2 | 0.4 | 0.02 | 0.06 | 0.2 |
| **CV (%)a** | 3.3 | 4.6 | 3.3 | 3.2 | 1.2 | 1.7 |
| **Accuracy (%)b** | 4.5 | 0.8 | 1.2 | -3.5 | -6.6 | -1.8 |

**Table 3:** Reproducibility and accuracy of the method

CV = (SD/Mean) x 100%

[{(Amount found)-(amount added)}/(amount added)}] x 100%

| Actual Value (µgmL-1) | THEOPHYLLINE | | | 1-METHYL URIC ACID | | |
|---|---|---|---|---|---|---|
| | 0.8 | 5 | 13 | 0.8 | 5 | 13 |
| **Mean Initial concentration (µgmL-1)** | 0.866 | 4.861 | 13.487 | 0.757 | 4.655 | 12.055 |
| **CV (%)a** | 2.16 | 4.1 | 0.26 | 2.24 | 6.30 | 0.29 |
| **Number of replicates** | 6 | 6 | 6 | 6 | 6 | 6 |
| **Mean final concentration (µgmL-1)** | 0.858b | 4.860b | 12.95b | 0.556c | 3.62c | 9.68c |
| **Recovery (%)d** | 99.07 | 99.97 | 96.01 | 73.44 | 77.76 | 80.29 |
| **CV (%)a** | 2.18 | 4.1 | 0.26 | 9.2 | 1.58 | 0.61 |
| **Number of replicates** | 6 | 6 | 6 | 6 | 6 | 6 |

**Table 4:** Stability of THEOPHYLLINE and 1-METHYL URIC ACID in plasma samples at -80$^0$C

CV = (SD/Mean) x 100%

Data obtained after 30 days

Data obtained after 1 days

[( Initial concentration)/(Final concentration)] x 100%

## Inter-Assay Precision:

As is apparent , inter-assay coefficients of variation determined from experiments performed on three days (n = 6) were <5%, this is indicative of good assay precision.

## Recovery:

Recovery was determined by dividing the peak area obtained from analysis of each of the two compounds added to plasma by that observed for the same amount of each compound injected directly into the chromatograph. Recovery of THEOPHYLLINE and 1-METHYL URIC ACID from plasma was 64 and 55%, respectively; these values were constant in the concentration range studied and are higher than those obtained in other studies[21, 22]. MonoacetylTheophyllinee was partially retained (11%) on the protein precipitate when trichloroacetic acid was used for deproteination[16]. This partially explains the low recovery of MonoacetylTheophyllinee.

| | |
|---|---|
| Mobile Phase | Acetonitrile (ACN): buffer (pH 3.0) (70:30v/v) |
| pH | 3.0 (± 0.05) adjusted with orthophosphoric acid |
| Flow rate | 1.0 mL/min |
| Injection volume | 25µl |
| Elusion type | Isocratic elusion |
| Column | X'terra RP18 (250*4.6) mm, 5µ |
| Temperature | 25 ±2 °C |

**Table 5:** Chromatographic Conditions.

## Stability:

Experiments conducted in our laboratory showed that QC solutions of Theophylline in plasma were stable for at least 30 days at −80°C; the amount of the initial concentration remaining after this time was 98.35 ± 2.07%. In contrast, loss of MonoacetylTheophylline in these plasma samples was substantial after storage at the same temperature for 1 day (a decrease to 77.16 ± 3.46% of the initial concentration).

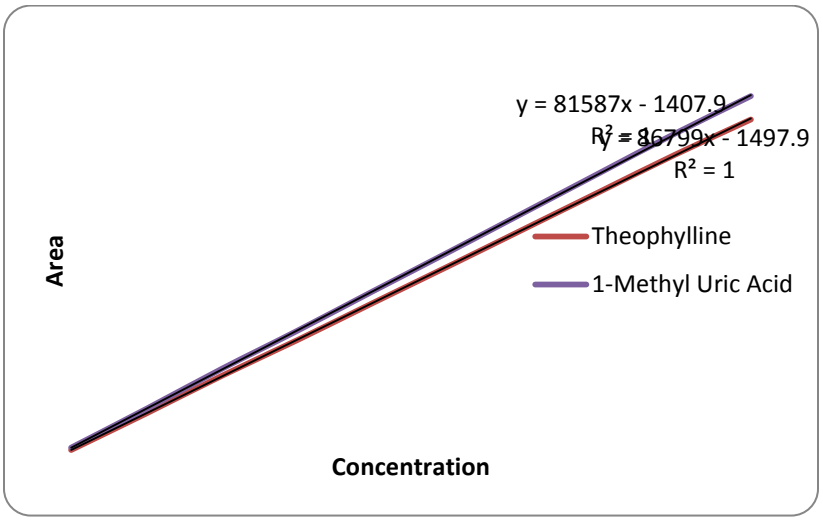


**Figure 1:** Linearity Plot of Theophyllinee and MonoacetylTheophylline

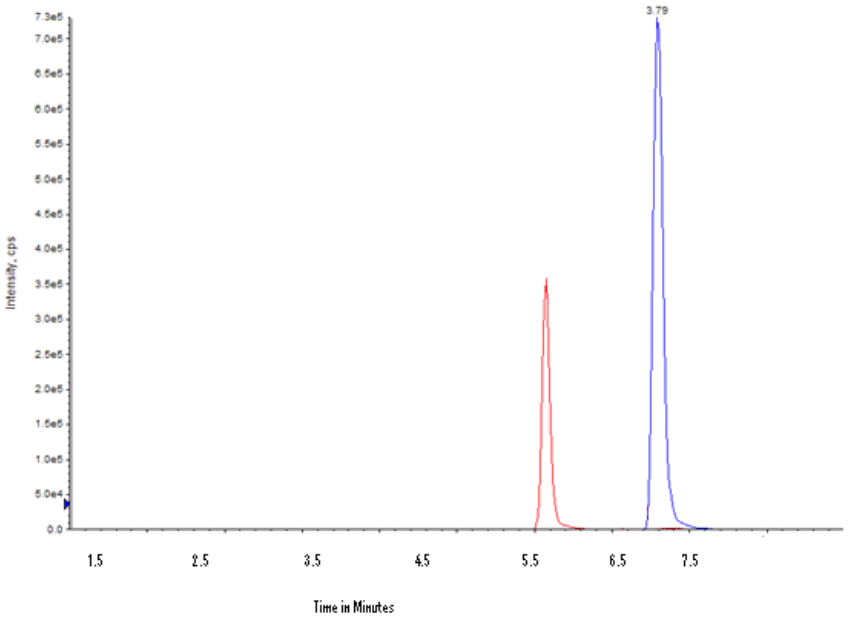


**Figure 2:** Peaks for THEOPHYLLINE and 1-METHYL URIC ACID

## CONCLUSION

The HPLC method developed for analysis of various formulations of Theophylline and 1-Methyl Uric Acid can be used for determination of Diclofenac in stratum corneum with the help of tape stripping method.

## REFERENCES

1. J N Pande, A Pande, and SPN Singh, "Acetylator status, drug metabolism and disease", The National Medical Journal of India. 16, 24-26 (2003).

2. Hombhanje F, "An assessment of acetylator polymorphism and its relevance in Papua New Guinea", PNG Med Jour .33, 107–10 (1990).

3. Drayer D E, and Reidenberg M M, "Clinical consequences of polymorphic acetylation of basic drugs", Clin. Pharmacol. Ther. 22, 251-258 (1977).

4. Serdula M K, and Rhoads G G, "Frequency of SLE in different ethnic groups in Hawaii", Arthritis Rheum. 22, 328-333 (1979).

5. Lunde P K M, Frislid K, and Hanstern V, "Disease and acetylation polymorphism", Clin. Pharmacokin.

2, 182-197 (1977).

6. Bouchardy C, Mitrunen K, Wikman H, Husgafvel-Pursiainen K, Dayer P, and Benhamou

7. S, "N-acetyltransferase NAT1 and NAT2 genotypes and lung cancer risk", Pharmacogenetics. 8, 291–8 (1998).

8. Gross M, Kruisselbrink T, Anderson K, Lang N, McGovern P, and Delongchamp R, "Distribution and concordance of N-acetyltransferase genotype and phenotype in an American population", Cancer Epidemiol Biomarkers Prev. 8, 683–92 (1999).

9. Parkes H G, "The epidemiology of the aromatic amine cancers. In: Searle CF (ed.). Chemical Carcinogens", Am Chem Soc Monograph. 173, 462 (1976).

10. Higginson J, "Chronic toxicology – an epidemiologist's approach to the problem of carcinogenesis", Essays Toxicol. 77, 29 (1976).

11. Evans D A P, and White T A, "Human acetylation polymorphism", J Lab Clin Med. 63, 394 (1964).

12. Ellard G A, "Variations between individuals and populations in the acetylation of Theophylline and its significance for the treatment of pulmonary tuberculosis", Clin Pharmacol Ther. 19, 610–25 (1976).

13. Weisburger J H, and Weisburger E K, "Biochemical formation and pharmacological, toxicological, and pathological properties of hydroxylamines and hydroxamic acids", Pharmacol Rev. 25, 1 (1973).

14. Evans D A P, Davidson K, and Pratt R T C, "The influence of acetylator phenotype on the effects of treating depression with phenelzine", Clin Pharmacol Therap. 6, 430 (1965).

15. Perry H M, Sakamoto A, and Tan E M, "Relationship of acetylating enzyme to hydralazine toxicity", J Lab Clin Med. 70, 1020 (1967).

16. Hughes H B, Biehl J P, Jones A P, and Schmidt L H, "Metabolism of Theophylline in man as related to the occurrence of peripheral neuritis", Am Rev Tuberculosis. 70, 266 (1954).

17. Woosley R L, Nies A S, Drayer D, Reidenberg M, and Oates J A, "Aceylator phenotype as a factor in procainamide-induced lupus erythematosus", Clin Res. 25, 279A (1977).

18. Wood A J J, and Zhou H H, "Ethnic differences in drug disposition and responsiveness", Clin. Pharmacokinet. 20, 350-373 (1991).

19. Reidenberg M M, and Martin J H, "The acetylator phenotype of patients with systemic lupus erythematosus", Drug Metab Disposition. 2, 71 (1974).

20. Gross M, Kruisselbrink T, Anderson K, Lang N, McGovern P, and Delongchamp R, "Distribution and concordance of N-acetyltransferase genotype and phenotype in an American population", Cancer Epidemiol Biomarkers Prev. 8, 683–92 (1999).

21. Kita T, Tanigawara Y, Chikazawa S, Hatanaka H, Sakaeda T, Komada F, et al., "Nacetyltransferase2 genotype correlated with Theophylline acetylation in Japanese tuberculous patients", Biol Pharm Bull. 5, 544–9 (2001).

22. Hildebrand M, and Seifert W, "Determination of acetylator phenotype in Caucasians with caffeine", Eur J Clin Pharmacol. 37, 525–6 (1989).

23. Relling M V, Cherrie J, Crom W R, Schell M, Mirro J, Meyer W H, and Evans W E, "Drug metabolizer phenotypes in American black irrvH.v white children (Abstract)", Clin. Pharmacol. Ther. 49, 173 (1991).

24. Jose M, Ladero, Jose F, Gonzalez, Julio Benatez et al, "Acetylator Polymorphism in Human Colorectal Carcinoma", Cancer Res. 51, 2098-2100 (1991).

25. Lee E J D, and Lim J M E, "A study of acetylator phenotype in normal subjects", Singapore Medical Journal. 22, 117-120 (1982).

26. Ellard G A, Gammon P T, and Wallace S M, "The determination of Theophylline and its metabolites acetylTheophylline, monoacetylhydrazine, diacetylhydrazine, isonicotinic acid and isonicotinylglycine in serum and urine", Biochem J. 126, 449-458 (1972).