DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR DETERMINATION OF THIOCOLCHICOSIDE FROM HUMAN PLASMA

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ABSTRACT

A stable, simple, rapid, precise, accurate HPLC method for analysis of Thiocolchicoside 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 Thiocolchicoside from Human plasma.

Keywords: Thiocolchicoside, HPLC, Validation.

INTRODUCTION

Thiocolchicoside (THC) is used clinically for its muscle relaxant, antiinflammatory, and analgesic properties, and it has been shown to interact with g-amino butyric acid (GABA) type A receptors (GABAARs) and strychnine-sensitive Glycine receptors in the rat central nervous system [1-6]. In contrast to a proposed agonistic action at these two types of inhibitory receptors, pharmacological evidence has shown that, under certain conditions, THC manifests convulsant activity in animals and humans [7]. Thiocolchicoside is originated from Flower, Seeds of Gloriosia superb ColchicaceaeGloriosa superb is the national flower of Zimbabwe (where it is a protected plant). It is also the state flower of Tamil Nadu state in India, and was the national flower of TamilEelam and as such was displayed during Maaveerar Day [8-12]. Thiocolchicoside is a natural derivated product from colchicine & a semi-synthetic derivative of colchicoside [13].

MATERIALS AND METHODS
Analytical Section

Extraction of Thiocolchicoside from plasma was achieved by a simple deproteination with trichloroacetic acid; this results in easy, rapid, and convenient separation of the analytes [14-18]. 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 Thiocolchicoside, 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 [19].

This HPLC method enabled rapid simultaneous measurement of Thiocolchicoside 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 Thiocolchicoside

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

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to be linear in the aforementioned concentrations. The correlation coefficient (r^2) of determination was 1 which indicates that the method is accurate.

Linearity

When average peak area was plotted against the Thiocolchicoside concentration 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; 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 Thiocolchicoside (three times the baseline noise) were 0.24 μg mL−1.

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).

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 the compound added to plasma by that observed for the same amount of each compound injected directly into the chromatograph [20-23]. Recovery of Thiocolchicoside from plasma was 64%, these values were constant in the concentration range studied and are higher than those obtained in other studies [24-25].

Stability

Experiments conducted in our laboratory showed that QC solutions of Thiocolchicoside 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%.

Table 1. Linearity of Thiocolchicosidee for HPLC method development

Sr. No.	Thiocolchicoside Concentration (ng/ml)	Area
1	1.004	68125
2	2.009	137259
3	3.013	208252
4	4.018	275685
5	5.022	345621
6	6.026	414865
7	7.031	485023
8	8.035	555210
9	9.04	625125
10	10.044	691242
	Slope	69425
	Intercept	-1198
	Correlation co-efficient	0.99999

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

Actual Value (μgmL-1)	Thiocolchicoside		
	0.8	5	13
Mean concentration found (μgmL-1)	0.9	4.5	12.7
Number of replicates	10	10	10
Standard deviation (SD)	0.04	0.3	0.6
CV (%)a	4.5	7.0	5.0
Accuracy (%)b	10.4	-9.8	-2.2

CV = (SD/Mean) x 100%

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

Table 3. Reproducibility and accuracy of the method

Actual Value (µgmL-1)	Thiocolchicoside		
	0.8	**5**	**13**
Mean concentration found (µgmL-1)	0.84	5.0	13.1
Number of replicates	6	6	6
Standard deviation (SD)	0.04	0.2	0.4
CV (%)a	3.3	4.6	3.3
Accuracy (%)b	4.5	0.8	1.2

CV = (SD/Mean) x 100%

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

Table 4. Stability of Thiocolchicoside in plasma samples at -80^{0}C

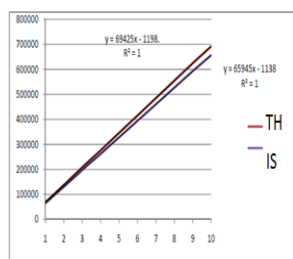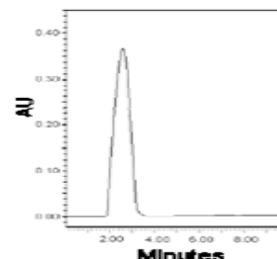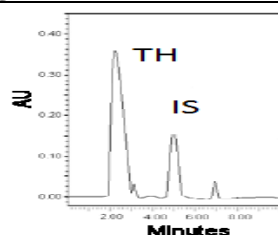| Actual Value (µgmL-1)	Thiocolchicoside		
	0.8	**5**	**13**
Mean Initial concentration (µgmL-1)	0.866	4.861	13.487
CV (%)a	2.16	4.1	0.26
Number of replicates	6	6	6
Mean final concentration (µgmL-1)	0.858b	4.860b	12.95b
Recovery (%)d	99.07	99.97	96.01
CV (%)a	2.18	4.1	0.26
Number of replicates	6	6	6

CV = (SD/Mean) x 100%

Data obtained after 30 days

Data obtained after 1 days

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

Table 5. Chromatographic Conditions

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

Figure 1. Linearity Plot of Thiocolchicosidee	**Figure 2. Peak for blank plasma**
Fig 3. Peaks for Thiocolchicoside	**Fig 4. Zero Interference Peaks for Thiocolchicoside**
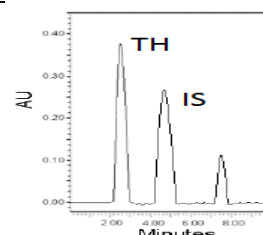
CONCLUSION

The HPLC method developed for analysis of various formulations of Thiocolchicoside can be used for determination of Thiocolchicoseide in Human Plasma.

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