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Original Article

QUANTITATIVE ANALYSIS OF CYTISINE IN *THERMOPSIS ALTERNIFLORAE* USING HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY

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ABSTRACT

Objective: An optimized high-performance thin-layer chromatography (HPTLC) method has been established for the quantification of cytisine in *Thermopsis alterniflorae* Regel and Schmalh.

Methods: Alcoholic extract of the aerial parts were prepared using Soxhlet extraction method. Separation was achieved on silica gel 60 F_{254} HPTLC plates using toluene-ethyl acetate-diethyl amine (7:2:1, v/v) as the mobile phase. The quantitation of cytisine was carried out using densitometric scanning at 545 nm after derivatization using Dragendorff's reagent.

Results: The linear regression analysis data for the calibration plot showed a good linear relationship (r^2 = 0.9849) in the concentration range 10 to 15 µg/spot. The method was validated for precision, repeatability, accuracy, specificity, limit of detection and limit of quantification. The average recovery was 99.0899% indicating good accuracy. The percentage yield of cytisine obtained was 0.5075+0.0135 % w/w.

Conclusion: The proposed HPTLC method was found to be simple, sensitive, accurate, reproducible, and robust.

Keywords: *Thermopsis alterniflorae,* Cytisine, High-performance thin-layer chromatography

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INTRODUCTION

Thermopsis alterniflorae Regel and Schmalh. (Fabaceae) is a perennial, erect, branched herb native to middle Asia, Russia, Kazakastan, Kyrgystan and Uzbekistan. The plant is documented to possess expectorant, vermifuge and hypolipidemic properties [1, 2]. Chemical investigations revealed the presence of alkaloids (cytisine, methyl cytisine, thermopsine, alteramine, dimethamine, pachycarpine), flavonoids (crotonoyl thermopsoside, crotonoylcosmosiin, formononetin, chrysoeriol, apigenin, luteolin, thermopsoside and cynaroside.), organic acids, sugars and resin in epigeal parts [2-7]. Cytisine is one of its important alkaloids used for acute respiratory problems and withdrawal of smoking cessation [1, 8, 9].

The present study is the first attempt to develop a validated HPTLC method for the quantification of cytisine in plant *T. alterniflorae.*

MATERIALS AND METHODS

Plant material and chemicals

Fresh, fully-grown, flowering plants of *T. alterniflorae* were procured from Uttarakhand and they were authenticated by a qualified taxonomist. A voucher specimen is maintained at the Department of the authors. The plant material was cleaned, dried, powdered to 60 # and used for the present study. Standard cytisine was procured from Sigma Aldrich, India. All the solvents used were of chromatography grade and the chemicals used were of analytical (AR) grade.

Preparation of standard solution

A stock solution (1000 µg mL^{-1}) of cytisine was prepared by dissolving accurately weighed 10 mg in 10 ml methanol in a volumetric flask. Standard solutions for calibration were prepared by dilution of the stock solution with methanol; the concentrations were such that amounts of cytisine between 8 to 16 µg.

Sample preparation

5 g plant powder was exhaustively extracted by refluxing with 100 ml methanol, concentrated and vacuum dried. The extract was dissolved in methanol in a volumetric flask to get the test concentration of 200 mg mL^{-1}. 3 µl of this solution was used for cytisine estimation. % of total alkaloids were estimated in the extract using Dragendorff's reagent [10].

Chromatographic conditions

HPTLC was performed on precoated silica gel 60 F_{254} plates (E. Merck, Germany). The plates were pre-washed by methanol and activated at 60 °C for 5 min. Samples were applied to the plates as bands 8 mm wide and 6 mm apart using Camag Linomat V applicator (Muttenz, Switzerland) fitted with a 100-microliter syringe (Camag, Switzerland). The linear ascending development was performed in Camag twin-trough glass chamber (10 × 10 cm) with mobile phase toluene: ethyl acetate: diethyl amine (7:2:1, v/v). After drying, plates were derivatized using Dragendorff's reagent and scanned in Camag TLC scanner using Win CATS software (version 1.4.3.6336) at 545 nm with slit dimensions 6.00 × 0.45 mm. The scanning speed was 20 mm/sec and the source of radiation was tungsten lamp.

Validation of the method

The method was validated as per the International Conference on Harmonization (ICH) guideline for validation of analytical method [11]. The intraday precision was determined by analyzing cytisine (10, 12, 14 µg/spot) for three times on the same day. The inter-day precision was determined by analyzing the same daily for three days. Repeatability of measurement of peak area was performed by measurement of the same spot seven times. For repeatability of sample application, the same volume of the standard solution was applied seven times and the area was measured. Accuracy of the method was ascertained by adding known concentration of analyte at three levels (80, 100 and 120% of working concentration) to the pre-quantified sample solutions and estimating the quantity of analyte using the proposed methods. LOD and LOQ were expressed as 3.3 a/b and 10 a/b, respectively, where 'a' is standard deviation of mean value and 'b' slope of calibration curve.

Quantification of cytisine

Three microliters of test solution (200 mg mL^{-1}) of the extract was spotted along with 8-16 µl of standard solution on plates for

estimation of cytisine. Peak areas were noted and quantification was performed using linear regression equations of respective compounds. A calibration curve was derived by plotting peak area versus concentration. The correlation coefficient, slope intercepts and regression equation were also calculated to estimate the mathematical estimate degree of linearity.

RESULTS AND DISCUSSION

Method validation

The alcohol extract (n=3) was found to contain 24.4+1.0334 % w/w residue. Total alkaloid content was found 9.15+0.845 % w/w. A series of standard solutions applied in the range 8-16 µg/spot was found to be having linear regression curve with r^2 value 0.9849 (fig. 1). The regression data obtained showed a good linear relationship. % Recovery of cytisine was found between 98.5668 to 99.6445% (table 1).

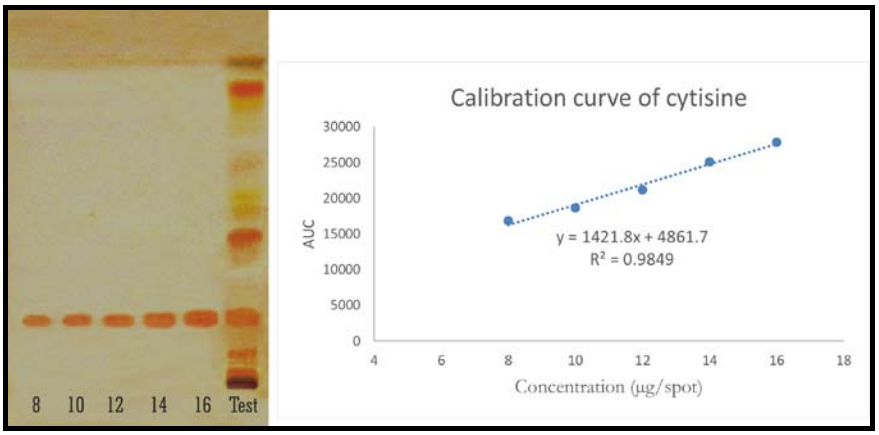

Fig. 1: HPTLC study of cytosine

Table 1: Recovery study results

Test	Std	Total	Avg (n=3)	Std dev	Co-var	% Recovery	% RSD
7.2874	5	12.2874	12.1113	0.1129	0.9324	98.5668	0.9190
7.2874	7	14.2874	14.1529	0.1453	1.0270	99.0586	1.0174
7.2874	9	16.2874	16.2295	0.0894	0.5512	99.6445	0.5492

Table 2: Summary of validation parameters for HPTLC of cytisine

Parameter	Result
Linearity (r^2)	0.9849
Range (µg/spot)	8 to 16
Precision (C. V.)	
Repeatability of Measurement	0.65
Repeatability of Application	1.031
Intra day	0.46 to 1.30
Interday	0.70 to 1.68
Accuracy (% recovery)	98.5668-99.6445
Limit of Detection (µg/spot)	0.6656
Limit of Quantification (µg/spot)	2.0101
Specificity	Specific

The data of Interday precision, intraday precision, and repeatability of measurement, repeatability of application, LOD and LOQ were given in table 2. The method was found specific as cytisine band was having no interference of other phytoconstituents in *T. alterniflorae*.

Quantification

Co-TLC of extract prepared and standard cytisine performed using toluene-ethyl acetate-diethyl amine (7:2:1, v/v) as mobile phase and silica gel 60 F_{254} as stationary phase showed cytisine at R_f 0.23 as orange colored spot in day light after derivatization with Dragendorff's reagent. UV overlay of the test was also observed to be similar with standard at 545 nm. The content of cytisine was found 0.5075+0.0135 % w/w in the plant.

CONCLUSION

A simple validated HPTLC method for the estimation of cytisine in *Thermopsis alterniflorae* has been developed. The established method is accurate, precise, reproducible and repeatable. The proposed HPTLC method can be used for routine analysis of extract or herb powder of *Thermopsis alterniflorae*.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Eisenman SW, Zaurov DE, Struwe L, editors. Medicinal plants of Central Asia: Uzbekistan and Kyrgystan. New York: Springer; 2013.
2. Khushbaktova ZA, Faizieva SK, Syrov VN, Yuldashev MP, Batirov ÉK, Mamatkhanov AU. Isolation, chemical analysis, and study of the hypolipidemic activity of the total flavonoid extract from *Thermopsis altherniaflora*. Pharm Chem J. 2001;35(3):155-8. doi: 10.1023/A:1010410029719.
3. Sabirov KA, Iskandarov S, Shakirov TT. Isolation of alkaloids from *Thermopsis alterniflora*. Chem Nat Compd. 1969;5(2):113-4. doi: 10.1007/BF00633303.
4. Shaimardanov RA, Iskandarov S, Yunusov SY. Alter amine-a new alkaloid from *Thermopsis alterniflora*. Chem Nat Compd. 1970;6:277.
5. Dobronravova EK, Sabirov KA, Shakirov TT. Polarographic determination of cytisine in *Thermopsis alterniflora*. Chem Nat Compd. 1970;6(1):74-6. doi: 10.1007/BF00564162.
6. Iskandarov S, Vinogradova VI, Shaimardanov RA, Yunusov SY. Dimethamine-a new bimolecular alkaloid from *Thermopsis alterniflora*. Chem Nat Compd. 1972;8(2):216-8. doi: 10.1007/BF00565303.
7. Yuldashev MP, Batirov K, Vdovin AD, Malikov VM, Yagudaev MR. Flavonoids of *Thermopsis alterniflora*: crotonoyl thermopsoside and crotonoylcosmosiin-New acylated flavone glycosides. Chem Nat Compd. 1989;25(3):303-8. doi: 10.1007/BF00597707.
8. Karnieg T, Wang X. Cytisine for smoking cessation. CMAJ. 2018;190(19):E596. doi: 10.1503/cmaj.171371, PMID 29759967.
9. Pastorino U, Ladisa V, Trussardo S, Sabia F, Rolli L, Valsecchi C. Cytisine therapy improved smoking cessation in the randomized screening and multiple intervention on lung epidemics lung cancer screening trial. J Thorac Oncol. 2022;17:1276-86. doi: 10.1016/j.jtho.2022.07.007, PMID 35908731.
10. Sreevidya N, Mehrotra S. Spectrophotometric method for estimation of alkaloids precipitable with Dragendorff's reagent in plant materials. J AOAC Int. 2003;86(6):1124-7. doi: 10.1093/jaoac/86.6.1124, PMID 14979692.
11. ICH. Q2 (R1) Validation of analytical procedures. In: Methodology in proceeding of the International Conference on Harmonization, Geneva; 2005.