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Research Article

DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR QUANTITATIVE ESTIMATION OF URSOLIC ACID IN LEAVES EXTRACT OF *MEMECYLON UMBELLATUM*

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ABSTRACT

A new validation and quantity evaluation method has been established by a defined high performance liquid chromatography by using Acetonitrile and water (80:20% v/v) as the mobile phase pumped through Phenomenex C_{18} (250×4.6 mm).The flow rate was 0.5ml/min and effluents were monitored at 210 nm.The retention time of ursolic acid was 12.26 minute. The linear regression analysis data for the calibration curve showed a good linear relationship with correlation coefficient of 0.9997. The concentration ranges were 20-100 mcg/ml. The limit of detection and limit of quantification were 0.419 µg/ml and 1.271 µg/ml respectively. The method is precise with relative standard deviation of 0.0905. The developed method has been validated for accuracy, precision, limit of detection, limit of Quantification, and linearity as per ICH guidelines.

Keywords: Ursolic acid, HPLC, ICH guidelines

INTRODUCTION

During the past decade, the therapeutic use of herbal medicine is gaining considerable momentum in the world[1]. Natural products are an important source of new structures leading to drugs in all major disease area[2]. *Memecylon umbellatum Burm*.F. (Memecylon) is a well known herbal drug in India. The leaves are used as astringent, antispasmodic, anti tumour and neuroleptic activities and also used to treat the leucorrhoea and gonorrhea[3]. The leaves and the barks are applied to bruises [4]. Further it also showed anti hyperglycemic and wound healing activity[5, 6]. Aerial parts was found to contain phytoconstituents such as umbellactone, β-amyrin,ursolic acid etc[7]. In the present study, a rapid and accurate method has been developed for the quantitative analysis of ursolic acid in the extract of *Memecylon umbellatum* as per ICH guidelines[8, 9].

MATERIALS AND METHODS

Reagents

Ursolic acid reference standard was purchased from Yacca lab Mumbai. Acetonitrile and water (HPLC grade) were purchased from MERCK specialties Pvt Ltd.(Mumbai, India)

Instrumentation

A shimadzu model HPLC equipped with quaternary LC-10 AVP pumps variable wavelength programmable UV/VIS detector SP10AVP column oven (shimadzu), SCL10AVP system controller (shimadzu), rheodyne injector fitted with a 20 µl loop and class- VP 5.032 software was used.

Chromatographic conditions

The chromatographic column used was a reverse phase C_{18}, 250 x 4.6mm, 5 mic Zorax RP-HPLC. The column and HPLC system were kept at ambient conditions. The mobile phase was acetonitrile: water (80:20% v/v) with the flow rate of 0.5 ml/min. The injection volume was 20 µl and elutes were analyzed at a wavelength of 210 nm. The mobile phase after filtration through 0.4 µm membrane filter was delivered at 0.5 ml/min for column standardization and baseline was continuously monitored during the process.

Preparation of standard solutions

Standard stock solution of ursolic acid was prepared by dissolving 10 mg of ursolic acid in 100 ml of methanol and various concentration of ursolic acid were prepared like 20,40,60,80 and 100µg/ml for calibration solutions. The calibration solution were analysed by HPLC and Calibration curve constructed by plotting the peak area of the standard against their respective concentrations.

Preparation of sample extract

Powdered leaves were macerated with methanol at room temperature for seven days. The solvent recovered by distillation in vacuum and residue stored was used for experiment.

RESULT AND DISCUSSION

Method Validation

The method was validated for accuracy, precision, Linearity and range, Limit of Detection and Limit of Quantification by following procedures.

Accuracy

The accuracy of the method was determined by recovery experiments. The Known concentration of working standard was added to the fixed concentration of the pre- analyzed extract solution. Percent recovery was calculated by comparing the area before and after the addition of the working standard. The recovery studies were performed in triplicate. The standard addition method was performed at 60, 80,100 µg/ml and percentage recovery was calculated. Recovery was within the limit of 100.2%.which indicated the method was accurate.(Table.1)

Precision

Test concentration utilized for intra-day and inter-day precision and results was shown in the table.1

Linearity and Range

The method was linear in the range of 20-100µg/ml for ursolic acid. The peak areas were recorded and calibration curve was plotted by peak area against concentration of drug.

Limit of Detection (LOD)

The detection limit (DL) was calculated by the following formula:

LOD= $3.3\sigma/s$;

Where

σ = the standard deviation of the response
s = the slope of the calibration curve.
The LOD was calculated as 0.419µg/ml.

Limit of Quantification (LOQ)

The quatification limit was calculated by the following formula:

Quantitation limit = $10\sigma/s$

The LOQ was calculated as 0.1.271µg/ml

Table 1: Optical characterization of ursolic acid

S. no	Parameters	Ursolic acid
1	Linearity range (µg/ml)	20-100
2	Correlation coefficient(r)	0.99969
3	Slope(m)	122.14
4	Intercept(c)	9769.4
5	Limit of detection (LOD ; µg/ml)	0.419
6	Limit of Quantification(LOQ ; µg/ml)	1.271
7	Tailing factor	1.10
8	Retention time (min)	12.26
9	Theoretical plates	3878
10	(%)R.S.D	0.2512
11	(%)Accuracy	100.2

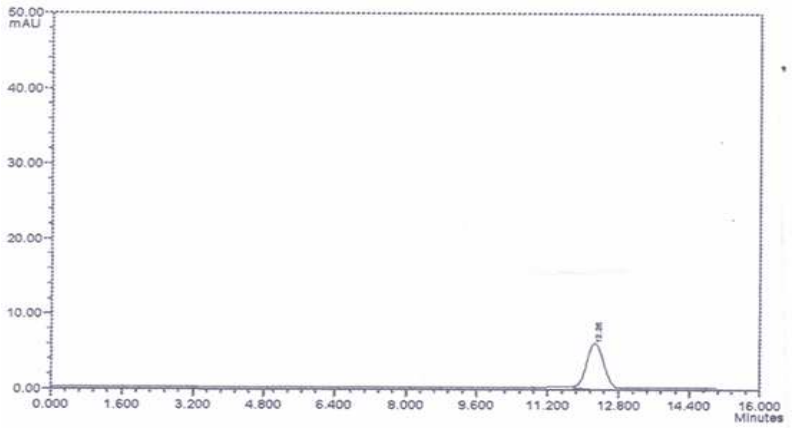

Fig. 1: chromatogram of standard ursolic acid.

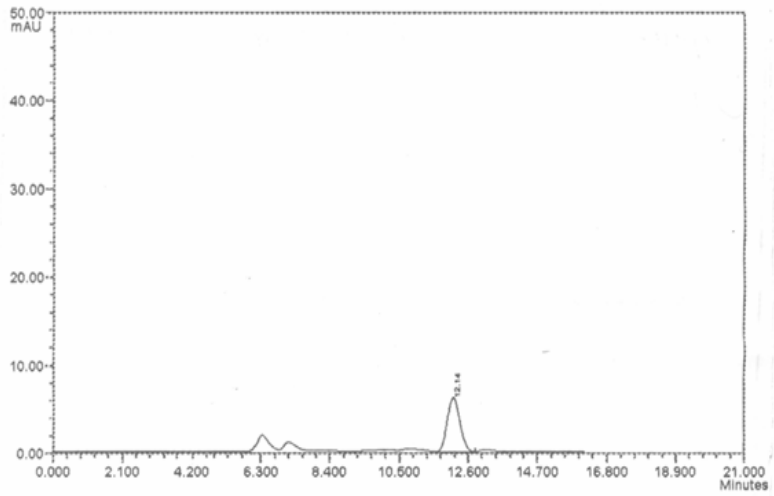

Fig. 2: Chromatogram of methanol extract of *memecylon umbellatum.*

CONCLUSION

The developed HPLC method was precise, specific and accurate for determination of ursolic acid in the methanol extract of plant leaves of *Memecylon umbellatum.* It is used in various diseases and very essential to develop a standardization method from which one can optimize its quantity in extracts as well as in its formulations.Running time and cost per analysis relatively low in comparison with other methods.

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