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DENSITOMETRIC HPTLC METHOD FOR ANALYSIS OF OLEANOLIC ACID IN *MENTHA PIPERITA* L

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ABSTRACT
A simple, monetary, reproducible, precise high performance thin layer chromatography (HPTLC) method was used to determine the content of oleanolic acid in methanolic extract of the aerial parts of *Mentha piperita* L. (Lamiaceae) using toluene: ethyl acetate: formic acid (4.0:1.0:0.1,v/v) as mobile phase. Quantification was performed by densitometry at λ = 529 nm. Oleanolic acid has been recognized to possess anti-inflammatory and antihyperlipidemic properties. In methanolic extract of the aerial parts, oleanolic acid content was determined to be 0.099 % w/w. The validated method showed linear response over conc. range of 500 ng to 5000 ng. The limit of detection was noticed to be of 2 ng and was statistically tested for repeatability by interday and intraday precision test as per ICH guidelines and its updated international convention. Method was found precise, accurate, specific and recoverable.

KEYWORDS: HPTLC-Densitometric determination. *Mentha piperita* L. Oleanolic acid. Triterpenes.

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INTRODUCTION

Oleanolic acid (3 -hydroxyolea-12-en-28-oic acid) is one of the most important phytoconstituent which is found in *Mentha piperita* L.(Lamiaceae) commonly known as peppermint. It is prescribed as aromatic stimulant and to treat spasmodic pain, indigestion, menstrual cramps, flatulence, nausea, gastroenteritis, insomnia, headache, colic, rheumatism, fever and cold[1]. The pentacyclic triterpenes are the common constituent of many medicinal herbs[2]. The traditional use of plants containing oleanolic acid in folk medicines are multiple including anti-inflammatory, hepatoprotection, analgesia, cardiotonic, sedative and tonic effects[3-5]. The pharmacological activities of this group of compounds are effective on chemically induced liver injury or parenchymal cell necrosis in rats[4]. Oleanolic acid occurs in many plants in the free acid form or as glycosides. The identification and quantification of oleanolic acid in natural extracts have been performed by HPTLC and LC-MS[6-7] and GC after silylation or methylation[8-9], Capillary supercritical fluid chromatography[10] and GC-MS after derivatization have also been used for plant analysis[11]. TLC method for qualitative determination of pentacyclic triterpenes was also described[12]. The present manuscript describes HPTLC method for analysis and quantification of oleanolic acid present in methanolic extract of the aerial parts of *M. piperita* with densitometric method.

MATERIAL AND METHODS

Chemicals & Reagents

Methanol, toluene, formic acid, ethyl acetate, concentrated sulphuric acid, ethanol acetone were purchased from S.D. fine chemicals, Mumbai.

Instrument

Sample was applied using a CAMAG Linomat-5 on 20

The plates were air dried and sprayed with10 % v/v sulphuric acid in ethanol, dried and then heated to130

Sonication of standard stock solution and concentrations

Oleanolic acid (standard) (25 mg) was purchased from Sigma Aldrich. It was accurately weighed dissolved in 50 ml of acetone and sonicated on ultrasonic water bath to dissolve it. The solution (0.5-32 µl) of this sample was applied using a CAMAG Linomat-5 on 20
foil sheet.

Sample preparation

The plant material (10 g) was air-dried at room temperature and exhustatively extracted with 200 ml methanol for 6 hours in a Soxhlet apparatus. The extract was dried on a steam bath under reduced pressure. The extract (10 mg) was dissolved in ethanol (5 ml) and filtered for analysis through a filter paper no. 0.44 mm.

Plant material

The aerial part of *M. piperita* L., were collected from the herbal garden of Jamia Hamdard and identified by Dr. H.B. Singh, National Institute of Science Communication and Information Resources (CSIR), New Delhi. A specimen sample no. NISCAIR/RHMD/Consult/-2008-09/1169/201 is deposited in the herbarium of NISCAIR.

Concentration of methanolic extract

The concentrations of methanolic extract were 0.5, 1.0, 2.0, 3.0 and 4.0 µl. The solutions were applied in a triplicate manner and spotted by automatic applicator.

Solvent systems

Chromatograms were developed using toluene: ethyl acetate: formic acid (4.0:1.0:0.1, v/v) ml as a developing solvent in a horizontal twin trough plate development chamber.

Specification of the plate
Silica gel 60F (254) precoated aluminium foil plate with thickness 0.2 mm (1.0554, Merck), were used as a chromatograph.

Track or concentration of the standard
15 tracks of standard having different concentration 500, 1000, 2000, 3000, 4000 and 5000 mg, of the standard were spotted on the plate in a triplicate manner.

Scanning
The densitogram of the extract and standard sample were obtained using a CAMAG Scanner 3. The measurement were carried out at

RESULT AND DISCUSSION
The standard sample of oleanolic acid (25 mg/50 ml) in different concentrations (0.5-35 µl) were applied in six bands to a plate and developed. The spots were derivatized, and the detector responses at different concentrations were measured at λ = 529 nm. A graph of peak area against amount of oleanolic acid was plotted. The mean, standard deviation, and % RSD were calculated. Intraday and Interday precisions were determined using oleanolic acid 500, 1000, 2000, 300, 4000 and 5000 ng/spot aliquots. The experiment was performed in triplicate. The data were analyzed by linear regression least-squares fitting.
Specificity of method was ascertained by analyzing standard oleanolic acid and extract. The spot for oleanolic acid in sample \was confirmed by comparing the Rf and spectra by overlaying oleanolic acid in extract to standard spectra of the spot with that of the sample. The peak purity of oleanolic acid was accessed by comparing the spectra at three different levels, i.e., peak start, peak apex, and peak end positions of the spot.
In order to estimate the limit of detection LOD and Limit Of Quantification (LOQ), blank chloroform was spotted six time.the signal to noise ratio was determined .LOD was considered as 2:1 and LOQ as 8:1 LOD and LOQ were experimentally verified by diluting known concentration of oleanolic acid until the average response were approximately 2 or 8 times the standard deviation the response for six replicate experiments.
Mobile phases of different composition were tested for HPTLC analysis of *M. piperita* L. sample and oleanolic acid in high resolution and reproducible results. The desired objective was achieved by use of toluene: ethyl acetate: formic acid (4.0:1.0:0.1, v/v) as mobile phase, which gave a peak at Rf 0.20 for oleanolic acid. **Fig. 1** shows the structure of oleanolic acid. HPTLC profiles obtained from the methanol extracts of M. piperita L. aerial part (leaves) and standard oleanolic acid In **Fig.2** oleanolic acid was linear in range 500-5000 ng per band with a correlation coefficient of 0.998.Y = 752(X)-302.6. Amount of oleanolic acid in methanolic extract of *M. piperita* L. aerial parts were found to be 0.099 % w/w. The method was validated by determining linearity, peak purity, repeatability and percentage recovery of oleanolic acid from samples (**Table1**).
The preliminary investigation for selecting suitable solvent system references has been given to those which are not too complicated in their composition, which possesses minimal temperature sensitivity and which gives exact and sufficient separation of constituents enough for a significant characterization of the drug. The methanolic extract of *M. piperita* L. has a very good separation on the selecting solvent system. The active constituent can be measured by determination of florescence and linear relationships between detector signal and compound concentration over the investigated concentrated range.
In thin layer chromatography, the chromatographic separation, which take advantage of the fact that different substances are partitioned differently between two phases during selection of the HPTLC results earlier reports , were also taken in to consideration. It was observed that HPTLC procedure for validation of oleanolic acid in the methanolic extract of *M. piperita* L. was simple, rapid and convenient.
The HPTLC method was the best for quantitative and qualitative analysis of pentacyclic triterpenes .The concentration of oleanolic acid in *M. piperita* L. was estimated to be 0.099 % w/w. The content of active

constituents was statistically calculated. This HPTLC method can be used to standardize the active constituent of the *M. piperita* L. and for investigation of natural drugs.

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Table 1: Peak areas used to construct the calibration curve for oleanolic acid, back-calculated concentrations and properties of the standard curve parameters for oleanolic acid

Content of oleanolic acid(ng)	Peak areas (mm×AU) (average)	SD(s) n=3	S D of mean S=s/√n	Lower 95% Confidence limit	Upper 95% Confidence limit
500ng	485.3433	0.6144	0.3547	26.344	29.396
1000ng	1159.67	0.9703	0.5602	43.693	48.514
2000ng	1900.217	0.1217	0.07024	80.318	80.922
3000ng	2777.733	1.060	0.6119	101.83	107.10
4000ng	3473.437	0.7306	0.4218	130.87	134.50
5000ng	4188.59	0.8534	0.4927	149.47	153.71

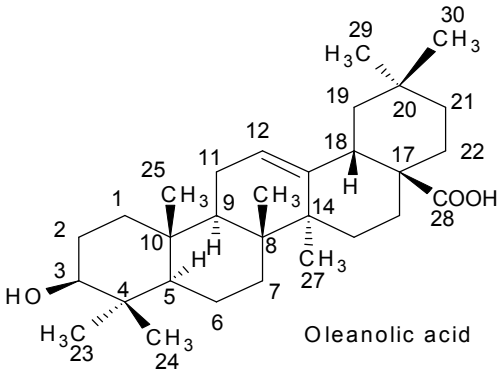

Figure 1: Structure of oleanolic acid

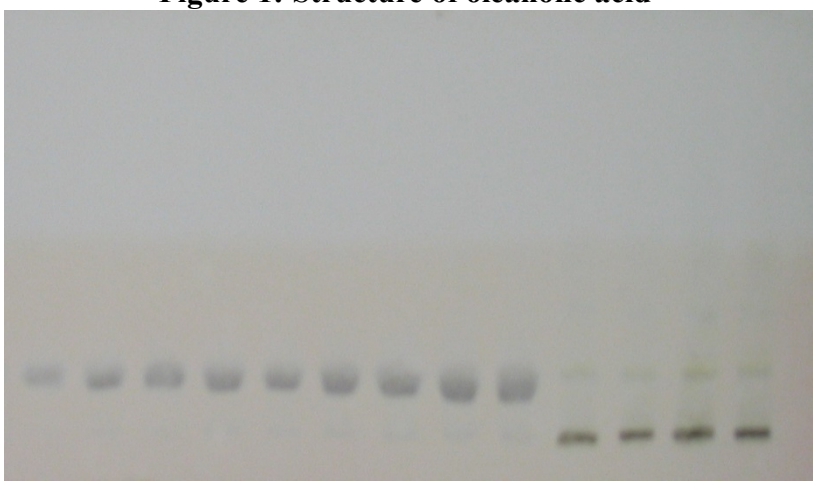

Figure 2: Documentation on day light .chromatogram of oleanolic acid in increasing concentration with crude methanolic extract of *M. piperita*. L. Solvent system-(toluene : ethyl acetate : formic acid, 4.0 : 1.0: 0.1)

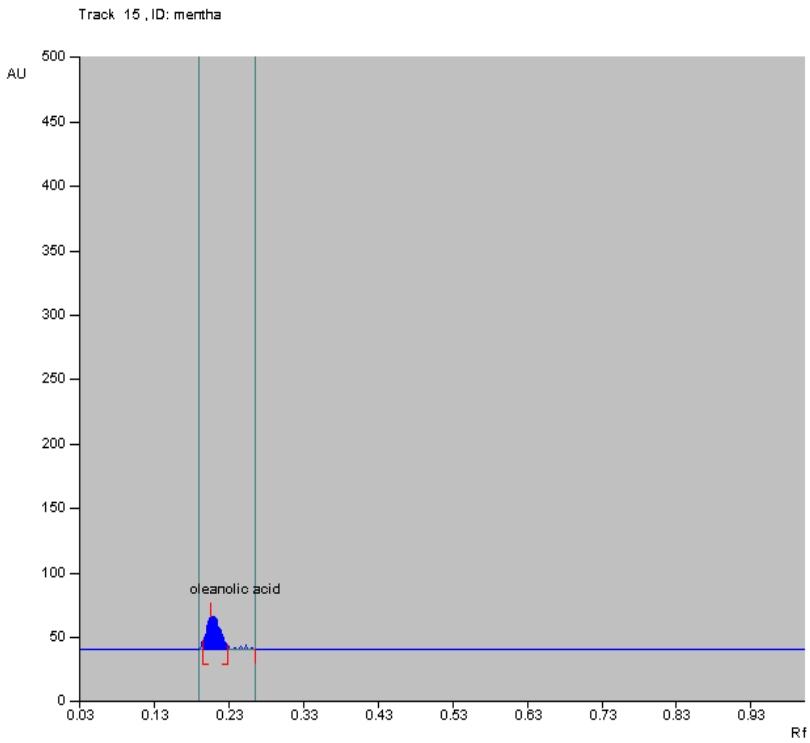

Figure 3: Peak of oleanolic acid in *M. piperita* L. methanolic extract determined at 529 nm

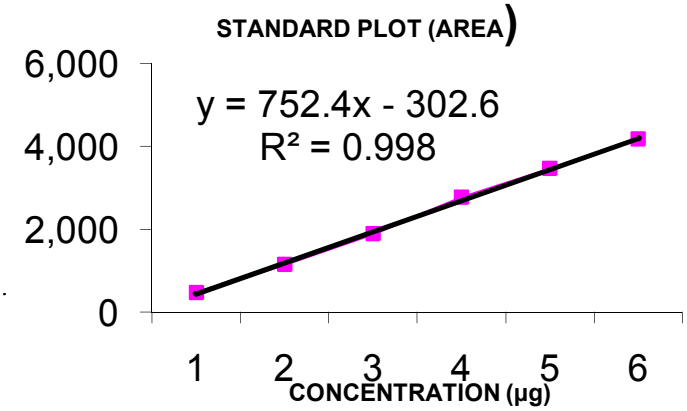

Figure 4: Calibration curve in between standard area and concentration

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