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RESEARCH ARTICLE

Stability Testing of Alliin by RP HPLC coupled with Electro Spray Ionization Tandem Mass Spectrometry in Unani Formulations

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ABSTRACT

Unani system is a science which deals with the preventive and promotive aspects of health of human beings and health problems occurred by the Ecological and Environmental factors, which may vitiate humours i.e. Blood, Phlegm, Yellow bile and Black bile, the fluids circulating in the body vessels. There is considerable demand in drug testing for a specific and precise analytical method for the identification of alliin in various Unani formulations. A combination of high performance liquid chromatography and mass spectrometry (LC-MS/MS) will provide unambiguous fingerprint information for estimation of alliin in Unani formulations. The objective of the present investigation is to develop a simple, economical and reliable high performance liquid chromatography method for the quantification of alliin in Unani formulations. The validated method allows quantification of alliin in 1 – 100.00 µg/mL. The correlation coefficient was ≥0.9990 for the Alliin. The simplicity of the assay and rapid liquid-liquid extraction make it an attractive procedure in estimation of alliin in Unani formulations. The separation was achieved in C18 column and positive ion mode was used for detection in ESI-MS detection.

KEYWORDS: Alliin; Imipramine and LC-MS/MS.

1. INTRODUCTION

Alliin [CAS No: Alliin (17795-26-5)], chemically (S-allylcysteine sulfoxide),is biosysnsethised from its parent compound S-Allyl cysteine and used demostrated to have lipid lowering, antioxidant activity, antibacterial/antifungal activity [1, 2, 3]. Liquid chromatography-electrospray-mass spectrometry (LC-ES-MS) has emerged as a sensitive and accurate analytical technique. Electrospray generates ions under atmospheric pressure and at relatively low temperature which minimizes thermal decomposition of labile compounds. In addition, mass spectrometry offers highly selective measurement by detecting specific mass-to-charge (m/z) ion related to analytical component; hence, more precise assignment of each eluted component. Various methods for estimation of alliin by spectrophotometry and HPLC has been reported [4, 5, 6, 7, 8]. The present paper reports a simple, precise and accurate method for the quantification of alliin by LC-ESI-MS/MS with positive ion mode in Unani formulations.

2. MATERIALS AND METHODS

Alliin [CAS No: (17795-26-5) (ALI-90.00%w/w) and Imipramine (CAS No: 50-49-7) (IMI-99.80%w/w) reference standard were a purchased from Sigma Aldrich, Bangalore and Varda Biotech., Mumbai, India respectively. ACE C18 RP (35 mm x 4.6 mm i.d., 3µ), ACE, USA was used as stationary phase. All chemicals and reagents used were of super gradient and purchased from Labscan Asia, Samutsakorn Province, Thailand. HPLC-grade water was prepared with a Milli-Q water purification system. A Thermo Finnigan (USA), HPLC system containing TSQ Quantum Discovery Max mass spectrometer (USA) was

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used for present study. Unani formulations were procured from Hamdard India Limited.

2.1 Preparation of reagents and solutions

Stock solutions of alliin and imipramine were prepared by dissolving weight equivalent to 2.50 mg in methanol and diluting upto 5.00 mL separately. Intermediate and working solutions were prepared by further diluting these stock solutions with methanol: water (40:60, %v/v). The mobile phase consisted of a mixture of methanol: 2mM ammonium acetate in water (50:50%v/v/v).

2.2 Sample preparation

The Unani formulations were extracted with ethanol: ethyl ether (1:1 ratio) by stirring at room temperature for 30 min. Extraction was repeated two times. The extracts were finally diluted with methanol in 1:10 ratio and filtered through a No. 1 filter. The solution was stored in the refrigerator (2-8 °C). A micro-porous filter (0.45 µm) was utilized to filter the solution prior to LC-MS/MS analysis.

2.3 High performance liquid chromatography and Mass spectrometric conditions

Chromatographic separation was carried out on a Thermo Finnigan HPLC with a ACE C18 RP, (35 mm x 4.6 mm i.d., 3µ) column. A mobile phase consisting of mixture of methanol: 2 mM ammonium acetate in water (50:50%v/v/v) was delivered with a flow rate of 0.600 ml/min isocratically. The total run time for each sample analysis was 3 min and column oven temperature was maintained at 40º C with injection volume of 20µL. Detection of alliin and imipramine was via LC--MS/MS.

The LC-MS/MS experiments were performed with a Thermo Finnigan LC module equipped with TSQ Quantum Disovery Max Triple Quads mass spectrometer in negative ionization mode. Nitrogen was used as a sheath gas at 50psi and argon was used as auxiliary gas at 20 psi. an electrospray voltage of 4500v was applied and the capillary temperature was set at 375°C. The mass analyzer was set to monitor positive ions. Alliin and imipramine were monitored at m/z 178.100> 86.100 and 281.200> 86.190 respectively at collision energy of 12 and 21v respectively (Table 1)

Molecule	Parent	Product	Width	Time	CE	Q1PW	Q3PW	Tube Lens
Alliin	178.100	86.100	0.500	0.200	12	0.70	0.60	100
Imipramine	281.200	86.190	0.500	0.200	21	0.70	0.60	90

Table 1: LCMS/MS Conditions

3. RESULTS AND DISCUSSIONS

A simple, specific, rapid and sensitive analytical method for the determination of alliin has been developed and used for stability studies.

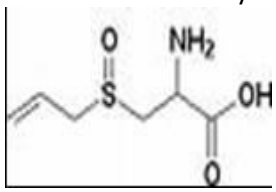

Figure 1: Structure of Alliin

Compound	Retention lime	Linearity range	r^2	Detection limit
Alliin	1.33	1-100 µg/mL	≥0.99	0.2 µg/mL

Table 2: Linearity Range with LOD

Compound	Amount spkied (µg/mL)	Recovery (Average of three results)
Alliin	10.0	98.60%

Table 3: Percentage Recovery

Product		Marker	Storage Conditions	% Degradation observed	
Lipotab	Lasuna	Alliin	25 ± 2°C/60± 5% RH	4.20	4.00

Table 4: Stability Result

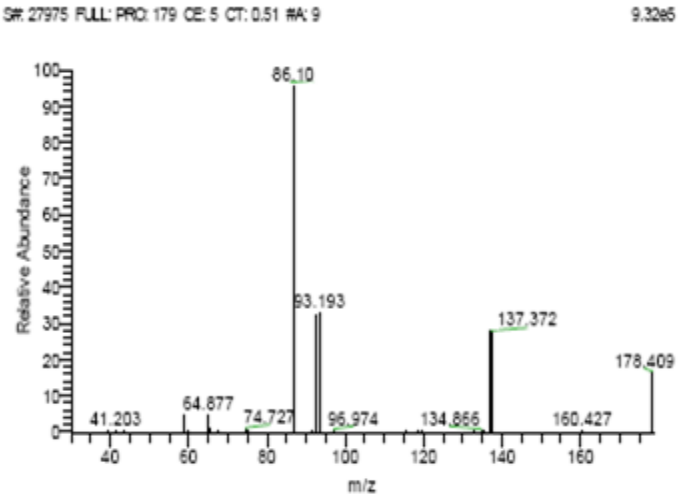

Figure 2: Representative positive ion spectra of Alliin

Electrospray is a soft-ionization technique, collision-induced dissociation (CID) has been used to enhance molecular fragmentation. Positive ion mode was employed for the detection of Alliin. The structure of the

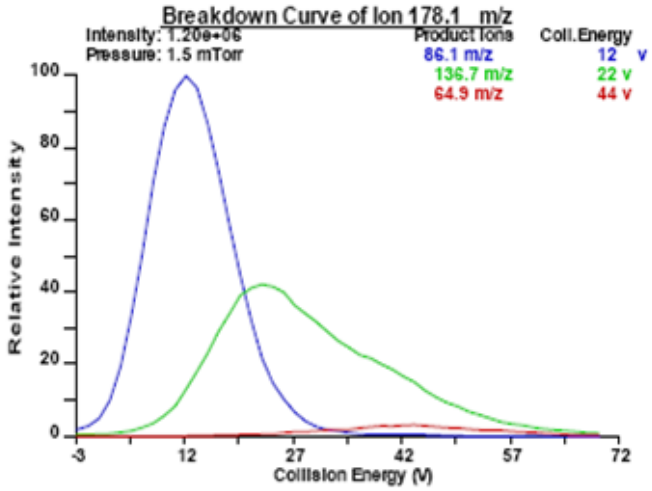

Figure 3: Breakdown curve of Alliin

alliin, positive ion spectra and breakdown curve of mass are captured in Figure1, Figure2 and Figure3 respectively. The linearity range with limit of detection is summarized in Table2. Adequate linearity ($r^2 \geq 0.9990$) was obtained through the range examined. The detection limit based on signal to noise of 5 was 0.5µg/mL in order to examine the matrix effect of the Unani formulation extract on determination of Alliin a known amount of pure alliin was spiked to see the percentage recovery which was found to be 98.60% which is summarized in Table3. It demonstrated that this sample extraction procedure is effective and indicated that there is no matrix effect on the measurement. Stability of alliin was measured using LC/MS-MS and the results demonstrated in Table 4.

In summary, this work has successfully demonstrated the potential of LC-ES-MS for quantitative determination of alliin and its use for stability studies in Unani formulations. Adequate linearity and detection limit were also obtained. In addition, the application of this newly developed method was demonstrated by analyzing Unani formulation samples. The other major advantage of this method over all those referenced is the short run time of 3.00 min with single step extraction technique as compared to already reported articles; LC-MS/MS method is a promising alternative to the analysis of alliin in Unanai formulations.

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Conflict of Interest: None Declared

