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Analysing Marigold Oleoresin Esters: Comparative Study between MALDI-TOF and LC-MS Chromatograms

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ABSTRACT

The present work details about comparing two mass analysers LC-MS and MALDI-Tof in analysing the marigold lutein esters. MALDI technique was found to be much simple, easier and quick without any sample preparation. Both LC-MS and MALDI-Tof were helpful in identifying all the expected fatty acid esters (FA1-lutein-FA2) from the oleoresin. This study in particular explores the possibilities of using MALDI-Tof in analysing the fatty acid esters of carotenoids from natural products.

Key words: *Lutein esters, MALDI-Tof, LC-MS, Mass spectrum, Comparison.*

INTRODUCTION

In the recent years, chromatography has evolved in manifold manner. With the advent and application of more powerful chromatographic and detection technologies, the isolation and characterization of even more minor carotenoid constituents in marigold oleoresins and other plant extracts has become possible. Different chromatographic and mass spectrometric techniques like Liquid Chromatography- Mass spectrometry (LC-MS); Gas Chromatography-Mass Spectrometry (GC-MS) and the recent Matrix Assisted Laser Desorption-Ionization (MALDI) have been developed. With such advents comparative studies are being made to identify the suitability of instruments which are designed for specific purpose. For e.g. LC-MS is used for studying carotenoids, flavonoids and other non-volatile and thermo labile organic compounds. Similarly, GC-MS is used for studying the composition of essential oils, perfumes and other volatile organic compounds. Ample literature is available pertaining to principle, feasibility and application of LC-MS, GC-MS and HPTLC. In the present study, the carotenoid profiling of marigold oleoresin is done using LC-MS and MALDI techniques and chromatograms obtained were compared. MALDI is a soft ionization technique initially developed for macromolecule analyses, which is greatly expanding due to its advantages, although more research is required to understand the processes involved, primarily the reactions in the ionization steps and fragmentation, especially for methods with a high energy transfer[1,2].

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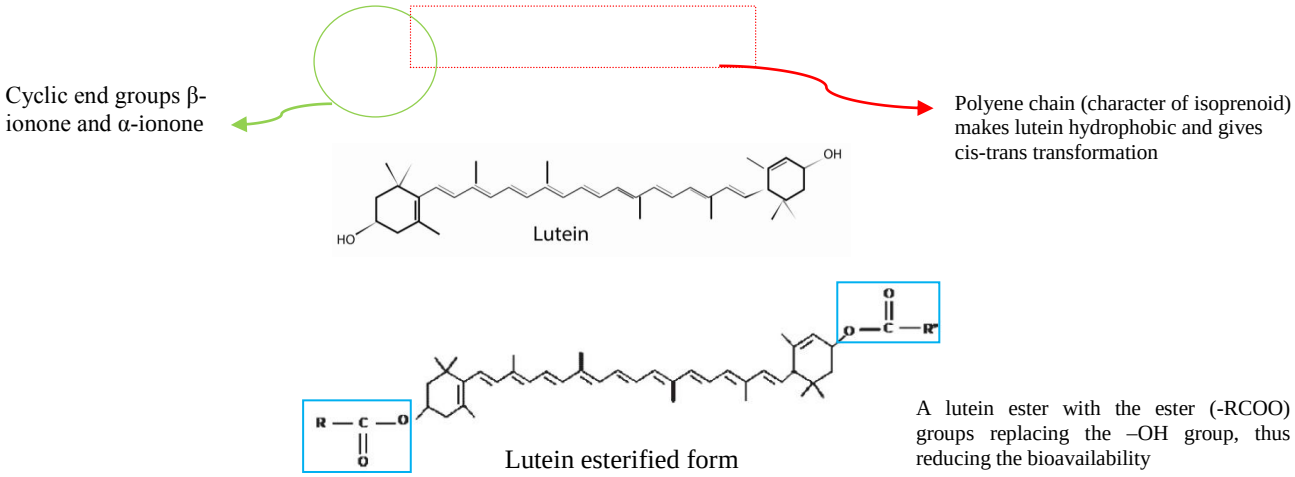
Lutein is an important member of carotenoid (xanthophyll) family widely present in marigold, spinach, kale[3] and has vivid applications from poultry to opthalmo-pharmacy industry. Scientific studies indicate that free lutein, unlike lutein esters, is the active compound in the human body that is deposited in the serum, eye and other tissues of the body and may be responsible for the reduction of risk of age-related macular degeneration (AMD) and increasing macular pigment density.



MATERIALS AND METHODS

Sample preparation: Dried marigold flower powder of DO-2 was used. As described by Tsao *et al*[4]., a 10 g amount of the powder was extracted four times with 100mL hexane (1:10, w/v) under constant stirring in RT and darkness. Each time the mixture was filtered through a Whatman No. 1 filter paper (Whatman, Maidstone, UK), under vacuum and all the filtrates were combined and concentrated to dryness in reduced pressure at ≤40°C in dark. The crude extract (oleoresin) was then reconstituted in HPLC grade ethyl acetate at a concentration of 10 mg/mL (1000ppm) and filtered through a 0.22 µm Whatman syringe filter maintained as stock solution. Before going for UPLC-HRMS/MS analysis the stock solution was then diluted to 10ppm with methanol and this solution was injected.

LC conditions: Thermo Scientific Exactive UPLC with PDA detector coupled with Thermo Scientific orbitrap Exactive HRMS/MS system having a quaternary pump, a degasser, a thermostatic auto-sampler and a DAD system was used for identification of lutein and its esters in the extract. Separation of lutein esters was carried out in a Kinetex C18 (50mm x 2.11mm id.; particle size, 5µm) column. The binary mobile phase consisted of water + 10mM ammonium acetate (solvent A) and methanol + 10mM ammonium acetate (solvent B). All solvents were filtered through a 0.45µm aforementioned syringe filter prior to analysis. The flow rate was kept constant at 400µL/min for a total run time of 40 min. The system was run in gradient mode:

Hold Time (min)	Mobile phase under gradient condition
0-24	70% A + 30% B
25-35	5% A + 95% B
36-40	70% A + 30% B

The injection volume was 20µL. The detector was set at 450 nm. Tentative identification of lutein esters was achieved by comparing their elution pattern with literature data, and by congruent UV–Vis spectra with that of authentic lutein standard.

Matrix assisted laser desorption ion time of flight mass spectroscopy (MALDI-Tof):

Marigold oleoresin lutein esters were analyzed using MALDI-TOF MS (AXIMA performance, Shimadzu, Kyoto, Japan) equipped with nitrogen laser 337nm, single photon energy of 3.6 V. Samples were deposited using a thin layer method, with the sample being deposited on top of the applied matrix layer on the target plate. Samples for MALDI analysis were first prepared by redissolving the oleoresin in 20µl ethyl acetate. To this solution was added 5-10 µl of ethyl acetate saturated with Dithranol matrix. A 5-10 µl aliquot of this mixture was pipetted on to the surface of slightly preheated sample holder, where the solvent evaporated within a few seconds. The standard lutein sample was coated with a saturated solution of CHCA0001 solution for obtaining the best elution of lutein.

RESULTS AND DISCUSSION

The lutein esters obtained from marigold oleoresin were separated and studied using LC-MS/MS and MALDI-Tof-Tof instruments. Almost a similar m/z spectrum was observed in both the mass spectra. As evident from Table 1 and fig. 1, a total of 8 important lutein ester peaks were identified viz., 762, 789, 961, 989, 1017, 1045, 1073 and 1101 in positive ion mode (M+H). From the previous literatures available that performed LC-MS-APCI for the marigold oleoresin, the obtained m/z values were correlated and the tentative/ probable diesters were obtained. From MALDI spectrum the peak 1045 was observed as the base peak and the relative concentration (%) was calculated. Two important ester peaks of m/z viz. 762 and 789 corresponding to lutein myristate-valerate and lutein palmitate dehydrate respectively were observed in abundance. In the latter case, the one end of lutein molecule seems to have underwent dehydration ($-1H_2O$) and this is the first incidence to be reported. The lutein diesters obtained insofar have not gone beyond those with known fatty acid patterns, i.e. C12/C14, C14/C14, C14/C16, C16/C16, C16/C18, and C18/C18[5].

The mass spectrum for the chromatogram obtained from the LC-MS/MS during RT 30.58-30.82min gave a further confirmation of the ester distribution (Fig. 2). The peaks with m/z in negative ion APCI mode 701.59, 761.85 and 785.67 were observed of which 785.67 was found to be novel and can be attributed to either an isotope replacement (C^{12}-C^{14}) or a lower order fatty acid moiety like valeric acid or caproic acid. In all it is very much evident that MALDI-Tof-Tof can be effectively used for fatty acid profiling in the marigold esters as substitute for LC-MS/MS. MALDI helps in the analysis of high-molar mass compounds by the combination of the analyte's solubilisation in an organic matrix and its excitation by a laser. In this process, the matrix must have a strong absorbance at a specific wavelength and must be easily sublimated. In the present study it is interesting to observe that the standard lutein was eluted best when using CHCA0001 matrix and hence confirming the mass of lutein at 569 (M_w+H) whereas in the sample it was best while using dithranol as the matrix. Such an observation is important in MALDI since the choice of matrix plays a major role. Different conventional matrices have been used for several purposes, such as DHB, CHCA, DHAP, SA, 4NA, THAP, Dithranol, nicotinic acid, picolinic acid, ferulic acid, and others, but few matrices were well characterized and many points are still unclear. The good features for matrices are linked to their solubility, absorptivity, reactivity, volatility, and desorption and a considerable number of reports provide details on preparation methods

of different matrices, which includes dried droplet, crushed crystal, fast evaporation, overlayer method, spin coating, and electrospray[6]. The use of DHB (2,5-Dihydroxybenzoic acid) as a matrix for carotenoids have been suggested by Fraser *et al*[7]., while performing MALDI-Tof-MS for *Lycopersicum* and Citrus juice extracts. In the present study DHB didn't produced a good spectrum as expected for the sample unlike dithranol and this can be hypothesized owing to the solubility and the nature of the sample (being a lipid)[6,8]. Tsao *et al*[4]., obtained a good separation of the lutein fatty acid esters from marigold oleoresins. Three major chromatogram fragments viz., $[M\text{-}FA1+H]^+$, $[M\text{-}FA2+ H]^+$ and $[M\text{-}FA1\text{-}FA2+H]^+$ were obtained by Tsao *et al*[4]., and this mass spectrum matched uniformly with the MALDI m/z spectrum (albeit the neutral loss of fatty acid peaks and the respective quasimolecular ion peak (lutein backbone in this study) peaks could not be obtained) obtained in the present study. Since diesters with identical fatty acids will only have two ions[9], in the present analysis the diesters with identical fatty acids were also obtained and the molecular weights obtained from MALDI matched with those of the LC-MS spectra. The higher molecular weight ester compounds could not be identified using LC-MS as the instrument was tuned using lutein standard (M_w= 568.17) thus limiting the spectrum detecting capacities to roughly m/z 500±300 units. Young *et al*[2]., also reported the use of LCMS for obtaining the marigold oleoresin ester composition. The fatty acids obtained in the present study through MALDI spectrum are in corroboration with those of Young *et al*[2]., who synthetically treated the pure lutein with different esters (Laurate, Myristate, Palmitate, and Stearate) as a means to confirm the presence of such high molecular lutein esters in marigold oleoresin. Presence of new and unidentified peaks in MALDI spectrum also confirms the influence of environment in deciding the marigold lutein ester composition[2,4,10,11]. In all, the present study confirms the use of MALDI-Tof MS as a means for obtaining the marigold oleoresin esters/ lipid composition in a similar manner as to that of LC-MS but in a much cheaper and time saving manner[6,12].

Table 1: MALDI-MS data of native lutein diesters from marigold oleoresin embedded in Dithranol matrix

Peak number (M_w+H)	Identity	Mw (m/z-1)	Lutein backbone	FA1	FA2
762	Lutein myristate	761	533	Myristate	Valerate
789	Lutein palmitate	788	533	Palmitate	Dehydro
961	Lutein laurate-myristate	960	533	Laurate	myristate
989	Lutein dimyristate	988	533	Myristate	Myristate
1017	Lutein myristate-palmitate	1016	533	Myristate	Palmate
1045	Lutein dipalmitate	1044	533	Palmitate	Palmitate
1073	Lutein palmitate-stearate	1072	533	Palmitate	Stearate
1101	Lutein distearate	1100	533	Stearate	Stearate

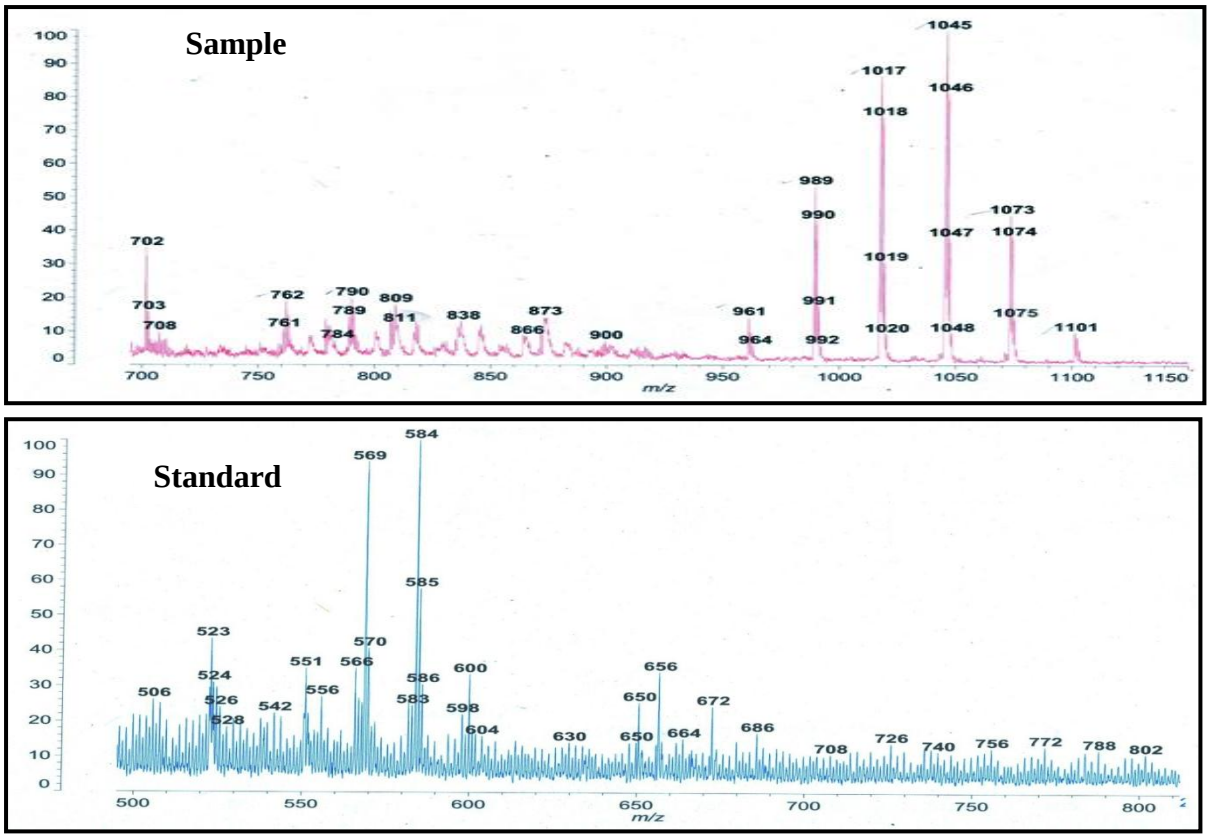


Fig. 1 a. MALDI-Tof/Tof mass spectrum of marigold oleoresin coated with Dithranol matrix b. MALDI-Tof/Tof mass spectrum of lutein standard coated with CHCA0001 matrix

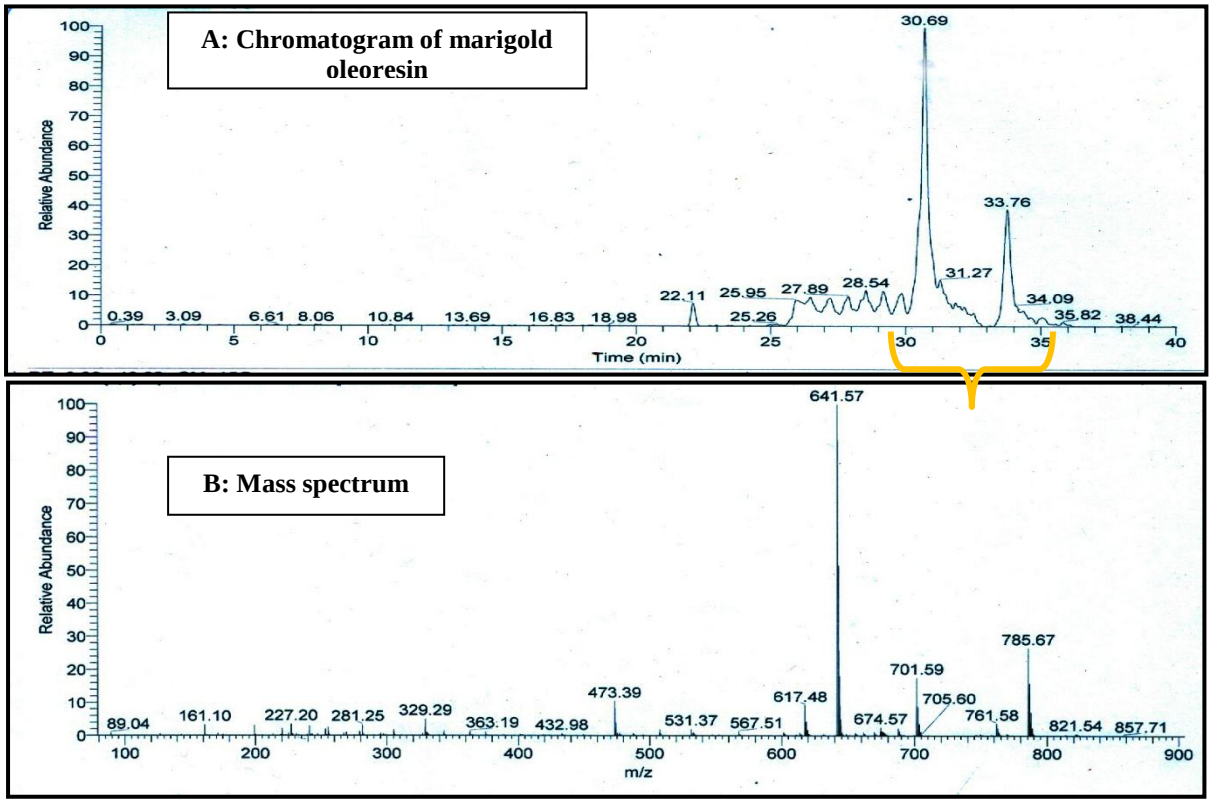


Fig. 2: Mass spectrum as obtained from negative ion UPLC-APCI-MS/MS for RT 30.58-30.82 from the chromatogram obtained for marigold oleoresin containing lutein esters

CONCLUSION

The present study confirms the application of MALDI-Tof in MALDI‑MS is an underexplored technique in natural products chemistry, but the advantages encourage its use in the field. Almost all the lutein esters ranging from 761 to 1100 molecular weights were identified in both the mass spectrometers. Its advantages include decreased ion suppression in complex mixtures, increased speed, and higher tolerance to impurities, increased sensitivity, and low sample consumption compared to LC-MS. Although several points are unknown in secondary metabolite analysis, MALDI has been successfully applied, thus demonstrating its potential and usefulness for analysing the marigold oleoresin.

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REFERENCES

1. Knochenmuss, R. and Zenobi, R., MALDI ionization: the role of in-plume processes. *Chem Rev*., **103:** 441–452 (2003).
2. Young, J.B. and Li, L., Impulse-driven heated-droplet deposition interface for capillary and microbore LC-MALDI MS and MS/MS. *Analytical chemistry*, **79(15):** pp.5927-5934 (2007).
3. Subczynski, W.K., Wisniewska, A., Widomska, J., Location of macular xanthophylls in the most vulnerable regions of photoreceptor outer-segment membranes. *Arch. Biochem. Biophys*., **504:** 61–66 (2010).
4. Tsao, R., Yang, R., Young, J.C., Zhu, H. and Manolis, T., Separation of geometric isomers of native lutein diesters in marigold (*Tagetes erecta* L.) by high-performance liquid chromatography-mass spectrometry. *Journal of Chromatography*, **1045(1):** 65-70 (2004).
5. Liu, H., Zhang, Y., Zheng, B., Li, Q. and Zou, Y., Microwave-assisted hydrolysis of lutein and zeaxanthin esters in marigold (Tagetes erecta L.). *International journal of food sciences and nutrition*, **62(8):** pp.851-856 (2011).
6. Silva, H.D., Cerqueira, M.A., Souza, B.W., Ribeiro, C., Avides, M.C., Quintas, M.A., Coimbra, J.S., Carneiro-da-Cunha, M.G. and Vicente, A.A., Nanoemulsions of β-carotene using a high-energy emulsification–evaporation technique. *Journal of Food Engineering*, **102(2):** pp.130-135 (2011).
7. Fraser, P.D., Enfissi, E., Goodfellow, M., Eguchi, T. and Bramley, P.M., Metabolite profiling of plant carotenoids using the matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *The Plant Journal*, **49(3):** pp.552-564 (2007).
8. Holscher, D., Fuchser, J., Knop, K., Menezes, R.C., Buerkert, A., Svatoš, A., Schubert, U.S. and Schneider, B., High resolution mass spectrometry imaging reveals the occurrence of phenylphenalenone-type compounds in red paracytic stomata and red epidermis tissue of Musa acuminata ssp. zebrina cv Rove Red'. *Phytochemistry*, **116:** pp.239-245 (2015).
9. Breithaupt, D.E., Wirt, U. and Bamedi, A., Differentiation between lutein monoester regioisomers and detection of lutein diesters from marigold flowers (Tagetes erecta L.) and several fruits by liquid chromatography-mass spectrometry. *Journal of Agricultural and Food Chemistry*, **50(1):** pp.66-70 (2002).
10. Bhattacharyya, S. and Dhar, P., Lutein from different cultivars of waste Marigold flowers: Extraction, Purification and Characterization by Chromatography & Mass spectrometry. *Indian Journal of Ethnophytopharmaceuticals*, **2(1):** pp 54-64 (2016).

11. Tian, Q., Duncan, C.J. and Schwartz, S.J., Atmospheric pressure chemical ionization mass spectrometry and in-source fragmentation of lutein esters. *Journal of mass spectrometry*, **38(9):** pp.990-995 (2003).
12. Wingerath, T., Stahl, W., Kirsch, D., Kaufmann, R. and Sies, H., Fruit juice carotenol fatty acid esters and carotenoids as identified by matrix-assisted laser desorption ionization (MALDI) mass spectrometry. *Journal of Agricultural and Food Chemistry*, **44(8):** pp. 2006-2013 (1996).