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APPLICATIONS OF ULTRA HIGH PRESSURE LIQUID CHROMATOGRAPHY TO NATURAL PRODUCTS

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ABSTRACT

Nature has afforded a number of interesting chemicals to be used in medicine, cosmetics, agriculture and others. The success in the studies of complex extracts of natural products depends on the development of new analytical techniques for the analysis of extracts. In view of increased separation efficiency, improved resolution and faster analysis time, Liquid chromatography established a method involving smaller particle size (<2µm) and high pressure (> 350bar). With the introduction of Ultra-High Pressure Liquid Chromatography (UHPLC), in 2004 new opportunities are emerging in the pharmaceutical industry for obtaining rapid analytical separations without sacrificing high-quality results in terms of resolution, accuracy, and reliability. To ensure the accurate quantification of the selected marker compound in natural products hyphenated techniques includes UHPLC-MS, SFE-UHPLC and UHPLC-DAD-ESI-MS. The present review seeks a newly developed analytical method as Ultra High Pressure Liquid chromatography together with the hyphenated techniques and its applications to natural products. This technique is very versatile and powerful tool for the separation of natural product from crude extracts for selective detection and general profiling. The technique is precise, robust, faster and sensitive and relies upon smaller volumes of organic solvents than HPLC. The advantages of introducing UHPLC are a decrease in sample turnaround time for both manufacturing and product development, the use of less organic solvents, and a reduction in generated waste.

Key words: UHPLC, natural product, hyphenated techniques, product development.

INTRODUCTION

Plants have always a rich source of active compounds which needs to be isolated for the activity. Natural products contain the active compounds which are complex structures and need to be separated. The separation can be achieved by using a well diversified analytical technique. Several analytical techniques have been employed for the separation of natural compounds like Spectrophotometric methods, Gas chromatography, Supercritical fluid chromatography, Capillary electrophoresis, High performance liquid chromatography, Ultra High Pressure liquid Chromatography. Ultra High Pressure liquid Chromatography is a developing technique based on the principle of separating the compounds based on increasing the resolving power of the analytical separation process particularly with the development of columns packed with porous sub 2micron meter particles used in very high pressure conditions. In this technique the use of smaller particle size results in high plate numbers as well as faster separations. This can be shown in Equation-1[1]

$$H = A + \frac{B}{\mu} + C\mu \qquad (1)$$

Since in the last eight years UHPLC technique is increasing widely for the separation as compared to HPLC technique because it eliminates complications resulting from use of larger samples volume, larger particle size, increased generated wastes and increased analysis time[2]. By comparing the intrinsic performance of such packing size with other intrinsic techniques such as monoliths, fused core technology or high temperature liquid chromatography with conventional particle size UHPLC with a maximum pressure of 1000bar is a very attractive strategy that

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generates a lowest analysis time for the separation. HPLC method is developed and validated for those plant product which are previously known and do not having co-eluting analytes but for newer plants extracts, there is need to develop a method which is having a greater resolution and lesser analysis time[3]. This review describes the use of Ultra High Pressure Liquid Chromatography (UHPLC) in analyzing natural products.

Since last five year's number of publications on the theory, principles, techniques and applications of UHPLC have been reported. UHPLC technique is considered as a valuable technique for the separation of natural products and can be coupled with various types of detectors for the various quantitative and qualitative analyses of natural products[4]. The application of this technique is in the field of herbal medicine and dietary supplement[4], food technology[5], explosive compounds[6], cosmetic products have been proved. By comparing its intrinsic performance with other existing techniques such as monoliths or high temperature liquid chromatography, it is very attractive strategy for improving chromatographic efficiency in the range 1000-80,000 plates[7, 8]. The tubing volumes, tubing length, gradient delay volume, column dimensions, mobile phase composition, injection volume, and detector cell volume are the parameters which are needed to be controlled for efficient separation. Improvements on these conditions have become the major themes in the development of UHPLC techniques.

Numerous studies for dealing with these problems have been published recently and many applications have been used to illustrate and validate the feasibility of the use of UHPLC technique.

A column with perfluorophenyl functionality helps to enhance retention and improve resolution when separating complex mixtures of substituted aromatic compounds[9.] The concept of making the column stable by using such a high pressure and the sample loading problem was solved using ethyl bridged hybrid particles for UHPLC column packing[10]. Analysis of substances with a wide range of polarities in a single run is estimated with ultra high pressure liquid chromatography composed of capillary packed column. The columns were packed with 1.5µm nonporous octadecyl silane modified silica particles[11]. To analyse and establish the impurity profile in the natural product, to decrease extracolumn band dispersion, to increase the mass loadability the smaller internal diameter of a column in mm is needed for proper separation[12]. A major application of this technique is in the estimation of natural compounds in traditional Chinese medicine (TCM). TCM has many complex structures containing natural compounds and metabolomics which are to be separated and for separation of these compounds a profound detector is must. Various hyphenated techniques is being developed are UPLC/MS-MS[13- 15], UPLC-PDA[16], UPLC- TOF-MS[17].

Apart from the separation method described above there are other methods for separation also, Liquid extraction method to separate flavonoids from plant extract using UHPLC method[18] proves to be significant among the separation. Separation of some compounds requires LC-MS method in conjunction with fast atom bombardment [19,] thermospray [20] and atmospheric-pressure ionization technique such as electrospray ionization[21-36] to avoid degradation due to GC-MS problems. Many papers have been published regarding the separation of proteins viz, High performance liquid chromatographic method, Ion exchange chromatography but the use of UHPLC method using monoliths column is also widespread in the field of separation of proteins by using UV detector [37]. For complex extracts different elution methods can be used to improve the resolution power significantly. These are classified as isocratic system, gradient system[38]. Simple UHPLC-UV[39] method, a UHPLC-TOF LC/MS[40] method for degradative samples of natural products has also used as a wide application in the separation of natural product. The content determination and fingerprinting analysis of TCMs can be performed efficiently by UHPLC technique for various active compounds[41].Various techniques used in pre and post column is often used to overcome the separation and detection of compounds [42].

NATURAL PRODUCT SEPARATION BY UHPLC METHOD

Gradient elution system is widely used in the separation of non polar as well as polar substituents in a compound. Separation of phenolic antioxidants and ascorbyl palmitate from edible oils is done on two columns. The sample of edible oils was eluted from the HPLC column C18 100 x 4.6 mm, 5 µm and analytical C18, 1.9 µm 50 mm x 2.1 mm column using a gradient system having Mobile phase A 70:30v/v, Acetonitrile and methanol and mobile phase B 1% phosphoric acid in water for HPLC

column and Mobile phase A 70:30v/v, Acetonitrile and methanol and mobile phase B 0.02% formic acid in water for UHPLC column. It was observed that 70% reduction in run time and 90% reduction in solvent usage were there by using UHPLC column as compared to HPLC column[43]. The separation is shown in Figure 1. By Liquid chromatography the profiling of crude extracts from plant origin or from other biological sources are easy to evolve into powerful tools for dereplication, quality assessment and metabolomics. Metabolite profiling of crude extracts can be easily done by high performance

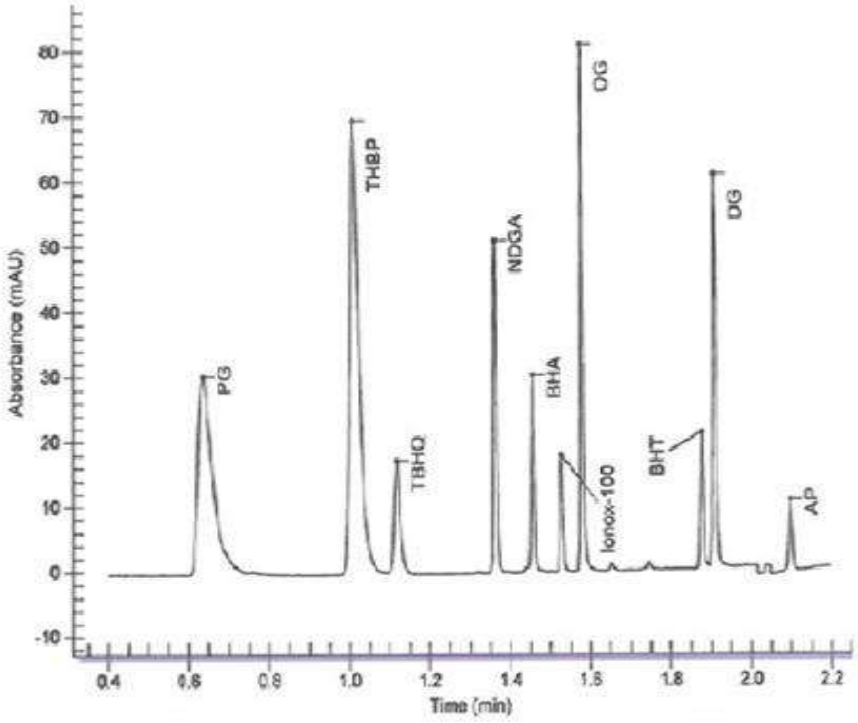

Figure 1 Separation of 10 antioxidants in edible oils viz., PG (propyl gallate), THBP (2, 4, 5-trihydroxybutyrophenone), TBHQ (tibutylhydroquinone), NDGA (nordihydroguairetic acid), BHA (2 (or 3)-tert-butyl-4-hydroxyanisole), OG (Octyl gallate), BHT (butylated hydroxy toluene), DG (dodecyl gallate), AP (Ascorbyl palmitate) by UHPLC method.

liquid chromatography (HPLC), such as liquid chromatography photodiode array detection (LC-PDA), mass spectrometry (LC-MS) or nuclear magnetic resonance (LC-NMR) [44].

The determination of neutraceuticals in natural compound by HPC method had a disadvantage of using larger run time and larger volume of mobile phase. This problem can be overcome by using UHPLC method. Determination of isoflavones in neutraceutical by UHPLC method is done by using 1.5µm particle 50 mm length C18 columns and at UV-Visible detector at 254nm[45].

The traditional Spectroscopic method for the screening of Xanthine oxidase inhibitor and superoxide anion scavenger is been widely used but using UHPLC-TQ-MS the estimation can be done in single analysis[46]. Detailed metabolite profiling and dereplication of crude plant extracts is mandatory for both Quality control and metabolomics purpose requires high resolution separation and sensitive detection and therefore to do so UHPLC-TOF MS detector was used at higher temperature. The percentage reduction was observed less as compared to the previous methods[47]. Conventional HPLC method provides insufficient resolving power for phenolics compounds due to the complex nature of natural compounds. So to overcome this process a new advanced UHPLC method was been developed and for the phenolic compound[48].

The development of high resolution methods related to HPLC for both chemical and biological profile has significantly increased the efficiency of classical bioactivity guided fractional procedures. A UHPLC method has been developed for the natural product drug discovery for neutraceuticals[49].

CONCLUSION

Ultra high performance chromatography has found its use for the separation of components found in natural products. The fact that UHPLC uses smaller particle size has decreased the analysis time for the separation is a big advantage that encompasses the problems of band broadening. Considering the savings of mobile phases the separation technique by using UHPLC method is being widely used in the future era of natural product evaluation.

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