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Investigation of porous graphitic carbon for triterpenoids and natural resinous materials analysis by high performance liquid chromatography hyphenated to mass spectrometry

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ABSTRACT

Natural resinous materials are mainly composed of pentacyclic triterpenes which exhibit a large number of interesting medicinal activities. However, the presence of numerous isomers within the active substances makes their screening by HPLC very challenging. Porous graphitic carbon was investigated as stationary phase to achieve triterpenes isomers separation. The influence of various parameters (temperature, formic acid concentration and mobile phase composition) on the retention was considered. A usual decrease of the retention of triterpenes was observed with the increase of the temperature. Therefore, separation in resinous materials was performed at 25 °C. Acetonitrile–isopropanol mixture was chosen as mobile phase in gradient elution and leads to the best compromise between efficiency and high resolution. The lack of chromophore groups in the pentacyclic triterpenes structures required the use of mass spectrometry detection. Moreover, atmospheric pressure photo-ionisation mass spectrometry prevents compounds fragmentation which was helpful for spectra interpretation and compounds identification.

1. Introduction

Pentacyclic triterpenes are found in plants especially in the bark of trees such as plane, cork, and birch but also in liquorice roots where they are particularly abundant. These compounds have been shown to exhibit significant biological properties for modern therapeutic drugs [1–5], for example A-ring or/and C-ring modified oleanolic acid derivatives were showed to have a significant anticancer effect on KB, MCF-7 and HeLa cell lines [6]. Other examples are the antitumour and anti-HIV activities of naturally occurring triterpenoids, including the lupane, ursane and oleanane scaffolds [7]. In the field of cosmetic industry, betulin derivatives have been patented as skin and hair protecting in compositions [8]. Moreover, triterpenes from natural products were recently used in anti-obesity therapy [9].

Triterpenes have been widely analysed and characterized using gas chromatography–electron impact mass spectrometry (GC–EI/MS) [10–20]. However, this method requires a time-consuming sample pre-treatment, which includes extraction, purification and derivatisation before injection into the gas chromatographic system. Thus, direct analysis of these compounds by liquid chromatography methods, such as liquid chromatography hyphenated to evaporative light scattering detector (ELSD) or HPLC–MS, reduces sample manipulation by avoiding the derivatisation step. That seems to be more suitable.

Previous work has succeeded in the separation of some pentacyclic triterpenes in reversed phase liquid chromatography using cyclodextrines in the mobile phase. However, considering the non-volatility of cyclodextrines, the use of ELSD required by the lack of chromophore was compromised [21].

In regard of literature, in one hand, the performance of reversed phase liquid chromatography is quickly limited regarding isomers separation, in the other hand, the porous graphitic carbon (PGC) stationary phase was showed to be a good alternative for isomers separation in reversed phase liquid chromatography leading to higher resolution of isomers molecules [22,23].

The high potential of PGC is due to its high hydrophobicity and polar and ionic interaction, caused mainly by the polarisable surface of the graphite [24–27]. These properties have been also used in supercritical fluids chromatography for the separation of different compounds types including isomers [28,29]. The separation of other isomers as polyglycerol fatty esters and fatty ethers [30], alkaloids [31], triacylglycerols [32] and polyunsaturated fatty acid methyl esters [33–35] were successfully attempted on PGC. Concerning triterpenes, the separation of only three acidic compounds (betulinic, oleanolic and ursolic acids) was described [36].

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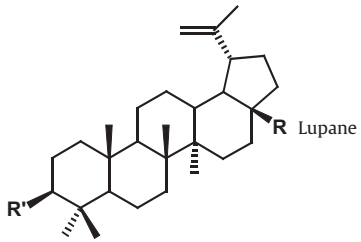
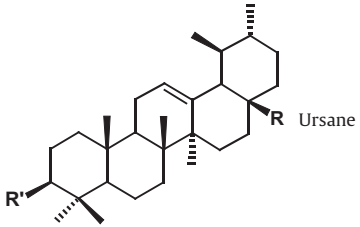
Table 1
Chemical structure of the studied pentacyclic triterpene standards [37].

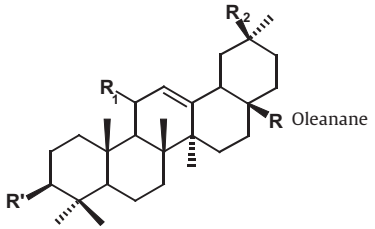
Structures of pentacyclic triterpenes



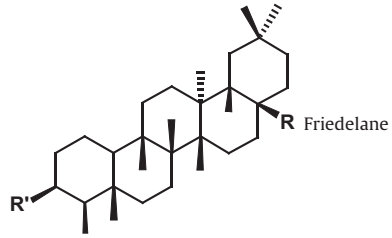
Lupane



Ursane



Oleanane



Friedelane

Compounds	Family	Substitution				
		MW	R	R′	R_1	R_2
β-Amyrin	Oleanane	426	—CH_3	α-H, β-OH	—CH_3	—H
α-Amyrin	Ursane		—CH_3	α-H, β-OH	—H	–
Friedelin	Friedelane		—CH_3	=O	—H	–
Lupeol	Lupane		—CH_3	α-H, β-OH	—H	–
Erythrodiol	Oleanane	442	—CH_2OH	α-H, β-OH	—CH_3	—H
Uvaol	Ursane		—CH_2OH	α-H, β-OH	—H	–
Betulin	Lupane		—CH_2OH	α-H, β-OH	—H	–
Oleanolic acid	Oleanane	456	—COOH	α-H, β-OH	—CH_3	—H
Betulinic acid	Lupane		—COOH	α-H, β-OH	—H	–
Ursolic acid	Ursane		—COOH	α-H, β-OH	—H	–