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Analytical methods to differentiate Romanian amber and Baltic amber for archaeological applications

Research Article

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Abstract: The study aims to establish several definite criteria which will differentiate Romanian amber and Baltic amber to certify the local or Baltic origin of the materials found in archaeological sites on the Romanian territory, by using light microscopy and performing analytical methods, such as Fourier transform infrared spectroscopy-variable angle reflectance and liquid chromatography with mass spectrometry detection. Experiments especially by Fourier transformed infrared spectroscopy, were applied to a wide range of samples with controlled origin. The methods were optimised and resulted in premises to apply the techniques to analysis of the archaeological material.

Keywords: *Romanian amber • FTIR-VAR • LC-MS • Light microscopy*

1. Introduction

Amber is a fossil resin originating from different types of Conifers and certain flowering trees, especially in hot climates. From the mineralogical point of view amber could be considered a mineraloid. There are many amber species and varieties; the most well known species are *the Baltic amber*, *the dominican amber*, the Sicilian amber named *simetit*, *burmite* - a resin extracted in the superior Burma and processed in China, the amber from Borneo, the amber from Alava (Spain) and the amber from the Romanian Carpathians named *Rumanite or Romanite* [1].

In Romania, spectacular amber has been exploited for a long time near Colti (Buzau County). The resin-bearing strata belong to the Oligocene in the Eastern Carpathian flysch. There are Palaeogene deposits belonging to the Colti facies (Palaeocene-Eocene) and the bituminous facies bearing Kliwa Sandstone (Oligocene). The resin-bearing strata are intercalated within the lower and medium part of the lower Kliwa sandstone (0.20-1.40 m). They consist of siliceous clay (0.20-1.40 m) always containing thin intercalations of bituminous shales (2-5 cm) and of preanthracite coal (1-2 cm). The paleobotanical and palynological researches on the amber-bearing formations give the age as Upper Rupelian-Early Chattian [2].

Romanian amber has been poorly analyzed by physical and chemical methods (especially in Romania) despite the fact that it is under assiduous attention of Romanian geologists, and data on archaeological amber from Romanian territory have not been provided to date. This was the reason to initiate the studies to establish certain methods for differentiating Romanian amber and Baltic amber. This is an important premise on which to draw firm conclusions about the origin of some artefacts found in archaeological sites from Romania.

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There are only a few literature data concerning chemical composition of Romanian amber (Romanite). An important study was carried out in 2000 by Stout *et al*. [3], reviewing geological and physico-chemical studies about this material [4-7]. In addition, according to data obtained by their own GC-MS and thermal pyrolysis analyses, they concluded that Romanite is thermally altered Baltic amber.

Recent studies have a similar goal with ours. In 2004, Shedrinsky *et al*. [8] presented a study based on pyrolysis-gas chromatography/mass spectrometry to differentiate six specimens of amber beads arising from an archaeological site, and in 2005, Angelini & Bellintani [9] published an article about European geological ambers of five different localities and types, and included Italian geological ambers from seven different deposits that were studied with Fourier transform infrared spectroscopy (FTIR) and diffuse-reflectance infrared Fourier transform (DRIFT). A classification of eight samples of amber of different geological age (Miocene to Triassic) and geographical origin is proposed by Tonidandel *et al*. [10] who employed direct mass spectrometric techniques, *i.e*. laser desorption ionization (LDI), atmospheric pressure chemical ionization (APCI) and atmospheric pressure photoionization (APPI), in order to obtain a fingerprint related to the amber origin. The best results are those obtained by APPI, indicating that the quantity of amber soluble components that can be photoionized decreases with increasing age, in agreement with the formation of highly stable, insoluble polymers. Guiliano *et al*. [11], used diamond crystal ATR FTIR spectroscopy to distinguish amber from immature resins such as copal, to determine local or Baltic origin of archaeological ambers and to detect most of the falsifications encountered in amber commercialisation. A systematic characterisation of Baltic amber, performed by FTIR, X-ray diffraction and scanning electron microscopy was recently reported by Pakutinskiene [12].

The aim of this work was to establish several definite criteria, which will differentiate the two types of amber to certify the local or Baltic origin of the materials found in archaeological sites on the Romanian territory, by performing analytical methods such Fourier transform infrared spectroscopy and liquid chromatography with mass spectrometric detection.

The study of natural polymers in thin sections using the polarizing transmitted light microscope is another important technique for the identification of the mineral inclusions in a sample and the textural relationships between them. This method also allows the investigations of the fluid inclusions and fossil animal and plants fragments.

An important fact to consider is that archaeological material is valuable and it is necessary to be preserved intact. Therefore Fourier transform infrared spectroscopy with variable angle reflectance was used to perform the analysis because it is a non-destructive technique. The second method employed, liquid chromatography with mass spectrometric detection was devoted to succinic acid (a ubiquitous component in amber) analysis. The sample lots used had controlled origins (known Baltic or Romanian origin) in order to establish some distinct patterns to discriminate between the two types of amber.

2. Experimental Procedures

Samples of Baltic amber and Romanite were studied as thin sections by transmitted light and microscopic techniques used in petrography. A Panphot Leitz Wetzlar microscope was used. The performance of this microscope permits an examination of a 4 cm^2 sample and the determination of fine particles down to 1-2 microns in diameter, such as fluid and solid inclusions (minerals, pollen, and spores). The thin sections were 0.03 mm thick. An automatic Nikon Eclipse camera E-400, 40 W was used for microphotographs.

Considering succinic acid as the specific water soluble component in the amber structure (historically known as spirit of amber or amber acid [13]) an HPLC-MS method was developed to assess its content. The sample quantities used for analysis were very small (1-2 mg). Samples were ground to powder by hand, into a porcelain mortar and extracted in acetonitrile acidified with formic acid (pH = 3.00), 1:1 ratio (mg amber/mL acetonitrile) at room temperature, without stirring, for 7 days. Note that samples of Romanite are easily ground while Baltic samples are harder and difficult to pulverize. The extracted fractions were filtered through 22 μm Millipore filters and analysed by LC-MS. The LC-MS experiments were carried out on a Shimadzu system equipped with two LC-20AD pumps, SPD-M20A photodiode array detector (operating between 200 and 600 nm) and coupled to a Shimadzu LCMS-2010EV MS detector equipped with an ESI interface. The separation was performed on a 50 × 2.1 mm Kromasil 100-3.5 C18 column, at 20^{0}C. UV and MS data were acquired and processed with a LC-MS Solutions operating system.

The mobile phase was: solvent A = 25% deionised water with formic acid, pH = 3.00; Solvent B = 75% acetonitrile with formic acid, pH = 3.00, isocratic (essentially from LaTorre *et al*. [14]). The flow rate was 0.1 mL min^{-1}. A negative ionization mode was used.

The nebulising and drying gas was nitrogen. The MS acquisition with the ESI interface was performed under the following conditions: probe high voltage, 1.5 V, nebulising gas (N_2) flow rate 0.18 L min^{-1}, curved desolvation line (CDL) temperature = 230^{0}C, acquisition mode SIM, searched ion $[M-H]^-$ = 117.

The run time was 15 min; 5 min of equilibration with mobile phase was required before the next injection. All the samples were analysed in triplicate, all the samples and mobile phase were filtered through 0.22 µm membranes. The quantification of the succinic acid from amber samples was performed on fortified samples.

FTIR assay was performed using FTIR-VAR technique with a beam incidence angle of 45^0, on a Bruker TENSOR 27 instrument. The samples were used without any pre-treatment, as whole pieces, fixed on a gold mirror, and all the spectra were registered *vs.* a background on clean gold foil. The resolution was 4 cm^{-1}, 64 scans were registered with an aperture of 4 nm. All experiments were performed in inert atmosphere (purging spectral purity Nitrogen) to acquire an appropriate resolution in the high wave-numbers (3200-3700 cm^{-1}) region. This is an important region for amber analysis, (the form and the frequency of the molecular absorption bands from this region are ascribable to the presence of carboxylic hydroxyls in free form or inter/intramolecular bonds).

3. Results and Discussions

Light microscopy analysis proves that Romanite has characteristic small surface cracks (Fig. 1) in comparison with the Baltic amber in which many fluid inclusions but no cracks were observed (Fig. 2). These facts demonstrate that diagenetic processes played an important role in the creation of Romanite. One of the most important consequences is the appearance of an internal organization tendency, proved by its weak optical anisotropy [15]. It may be assumed that the existence of cracks is a definite criterion for Romanite, probably as a consequence of its lower proportion of free water.

For the Baltic amber, in our opinion the fluid inclusions are a definite criterion for this type of amber.

Another consequence of diagenetic processes is represented by re-mineralization in Romanite (substitution of organic matter by anhydrite and feldspar) (Fig. 3). In other microscopic observations, the organic tissue was replaced by resin [16].

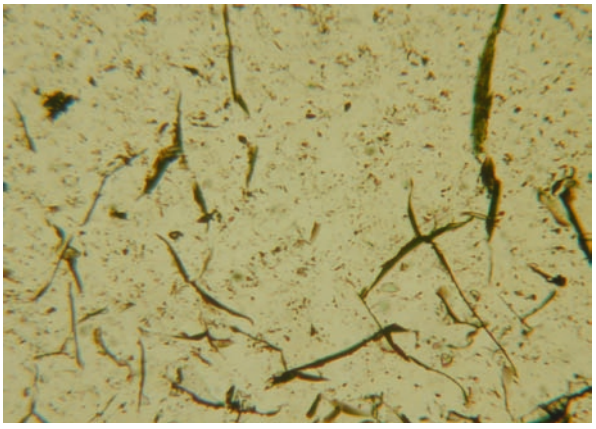

Figure 1. Surface cracks in Romanite, NII, 20x/0.33

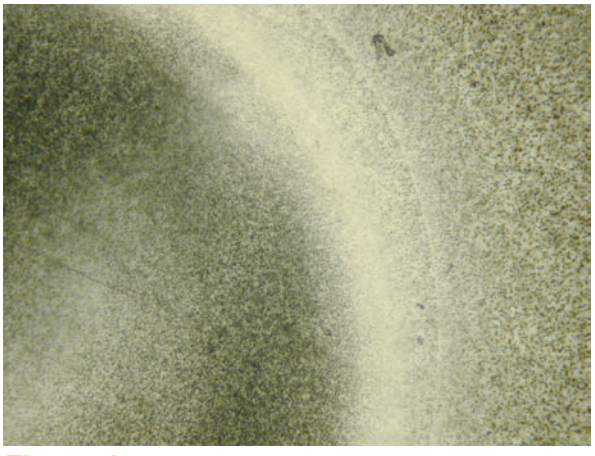

Figure 2. Fluid inclusions in Baltic amber disposed as concentric bands, NII, 20x/0.33

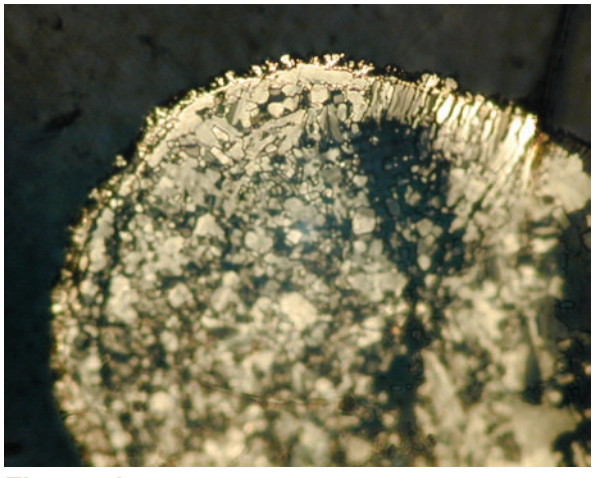

Figure 3. Organic spherules (pollen) in Romanite: the organic matter is substituted by feldspar, N+, 10x/0.25

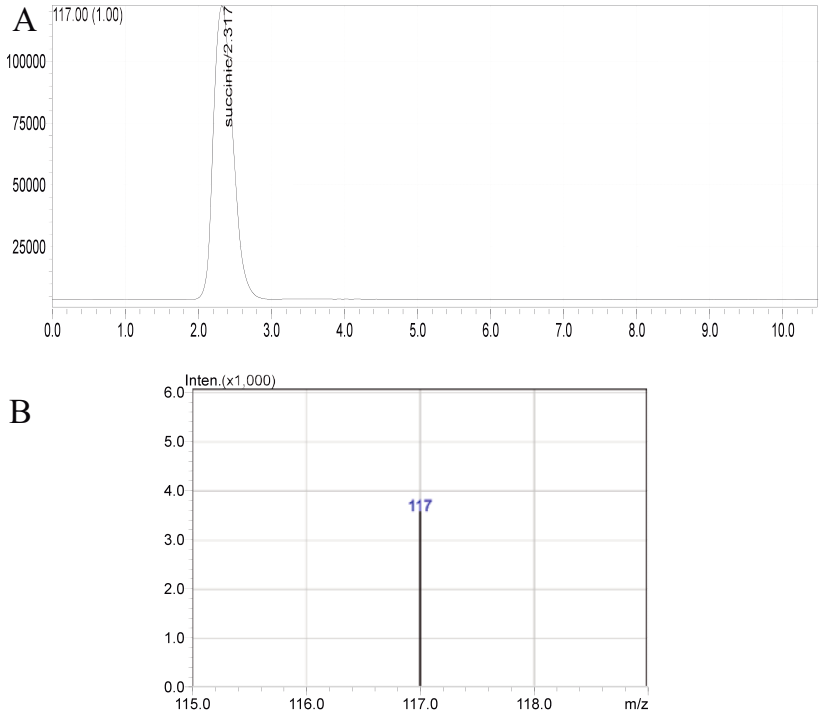

Figure 4. A) Chromatogram of standard (Sigma) succinic acid: 7.5 μg mL^{-1}; B) Signal intensity for succinic acid ion

3.1 LC-MS analysis

The concentration of succinic acid was determined by LC-MS method described above [14] using a calibration curve (Y = 284940.0X - 108595.5; R^2 = 0.9870; R = 0.9935). The linear range of succinic acid concentration levels was 0.1 to 10 µg mL^{-1}. The obtained detection limit was 0.085 µg mL^{-1}. Chromatograms of some representative samples selected from the 15 ones analyzed by this method are presented in Figs. 4 and 5.

The MS detection was chosen due to technique sensitivity (500 times more sensitive than UV detection) and in order to ensure the proper identification of the succinic acid from a sample with complex matrices. The retention time was identical for standard and samples. The quantitative results for succinic acid found in different samples (searched ion mass = 117, as seen in Fig. 4B) are presented in Table 1.

The LC-MS analysis performed on amber samples fortified with 1 µg succinic acid per mg sample, gave results of concentrations ranging between 0.27 and 1.11 µg mg^{-1} of succinic acid in Baltic samples and concentrations between 1.66 and 7.75 µg mg^{-1} in Romanite samples. Therefore, we could conclude that it is possible to distinguish Romanite from Baltic amber using the succinic acid determination by this technique: the quantities of succinic acid extracted under prescribed conditions being higher for Romanite samples (and for other species of younger resins, such as copal). The explanation could be that, under the extraction conditions employed, succinic acid is extracted more easily from less compact structures, and the extraction is more difficult from dense structures (like Baltic amber structure, older and with more bonds which must be broken). Perhaps the higher concentrations of succinic acid found in Romanite are also due to its easier extraction. As stated in the discussion on Figs. 1 and 2, Romanite has cracks in the surface (and probably throughout the whole sample, even when ground and consisting of fine particles). This should facilitate penetration with acetonitrile and lead to a more rapid and a higher degree of extraction of succinic acid. From all the tested Romanite samples only one exception was noted, that of sample 280 Romanite, which has a very low content of succinic acid.

In order to preserve archaeological materials (which are clearly and specifically addressed in our studies) the quantities of available sample are very small, maximum 1-2 mg. As a consequence a total extraction of the succinic acid from the samples cannot be performed, and we attempted to develop a procedure applicable to small amounts of amber. This is one of the reasons for which our results are not comparable with those published in the literature concerning the different ratios of succinic acid from different types of amber. The other

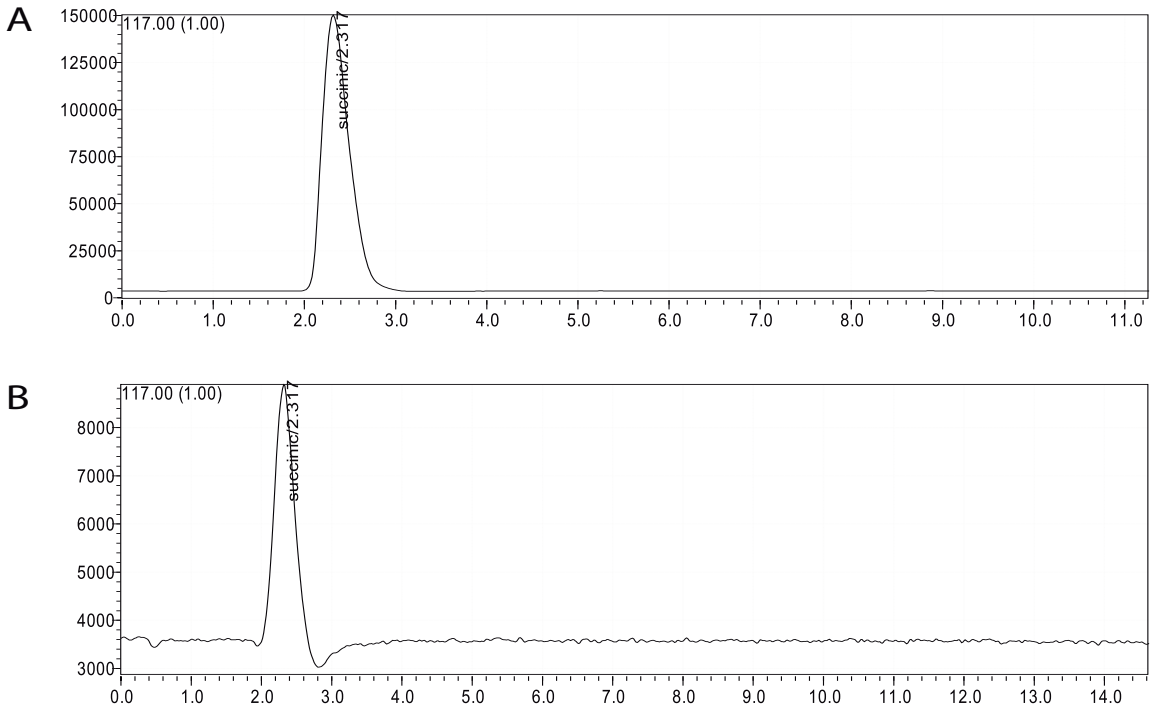

Figure 5. A) Chromatogram of sample 383 (Colti-Patarlagele); B) Chromatogram of sample 355 (Bitterfeld-Germany)

reason consists in the fact that we used unique samples (received from geological museums), of controlled origin but which had not been analysed to date. It should be emphasised that Baltic amber is known to contain about 3-8% succinic acid that is one of the main distinguishing properties of this fossil resin [17]. The differences in succinic acid contents of different samples could appear because amber is not a homogenous material. Still, these preliminary results are interesting, and rapid and simple LC-MS analysis, together with other methods, could bring a certain decision about the origin of the material.

Table 1. Composition in succinic acid obtained by LC-MS from samples with controlled origin (Baltic amber and Romanite).

No. Crt.	Sample ID	Succinic acid $\mu g\ mg^{-1}$ amber
1	211/Baltic	1.04±0.20
2	204/Romanite-Colti	7.19±0.08
3	209-copalit	4.42±0.15
4	301-Palanga Lithuania	0.43±0.03
5	280-Colti Buzau	0.19±0.05
6	321-Colti Patarlagele-gallery	6.77±0.06
7	306-Sibiciul de jos	1.45±0.11
8	329-Poland	1.11±0.08
9	341-Kaliningrad	0.27±0.05
10	355-Bitterfeld Germany	0.82±0.12
11	383-Colti Patarlagele	7.75±0.03
12	119-Colti	1.66±0.01

3.2 FT-IR –VAR analysis

24 samples from different origins were analysed by FTIR-VAR, 13 from Romania, 6 from the Baltic region (Palanga and Kaliningrad), 2 from Germany and 3 from Poland in order to settle a certain pattern to differentiate Romanite amber from other types of amber, especially the Baltic variety.

In the case of uncertain observations, to clarify some spectral zone from the fingerprint region (1300-900 cm^{-1}), such as in region 1450-1800 cm^{-1}, the transmittance spectra of the samples were used for comparison.

Spectra were analysed and assigned on the three wavenumber domains of interest for amber, namely those between 2000-3600 cm^{-1}, 1350-1820 cm^{-1} and the 1045-1250 cm^{-1} regions, which correlate with hydroxyl groups, carboxyl groups, carbonyl groups and with C=C unsaturation.

The working hypotheses were based on some observations, namely:

a. Regardless of the IR technique applied, there are no notable differences between Romanite spectra and Baltic spectra in the 3000-3600 cm^{-1} region. The notable differences appear in the 'Baltic shoulder' region, 1060-1250 cm^{-1}, and in the 1155-1161 cm^{-1} region. For the Baltic amber, the shoulder appears in the region 1275-1155 cm^{-1}, while that of Romanite is shifted to about 1045-1020 cm^{-1}, as may be noticed from Fig. 6.

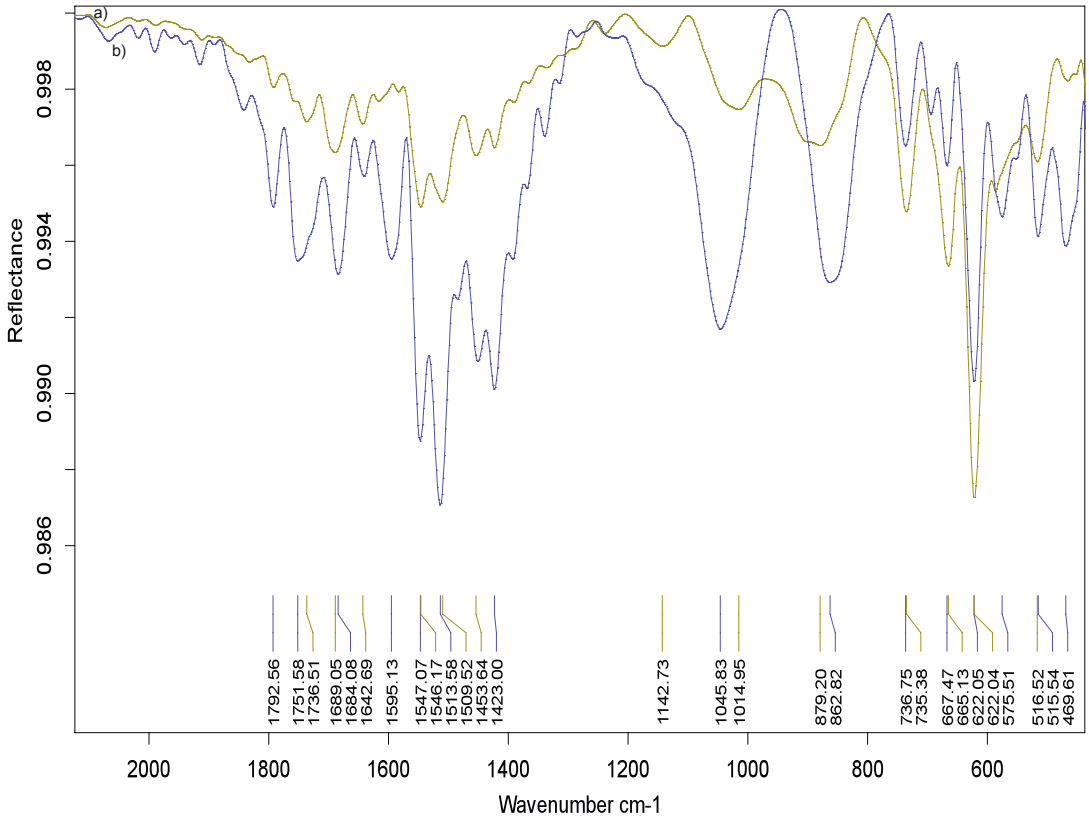

Figure 6. FTIR-VAR (45°) spectra comparison, region 600-2000, for a) Baltic amber (211____) and b) Romanian amber (204____)

b. An important difference in Variable Angle Reflectance spectra is registered for absorption bands from 900-800 cm^{-1} region (Fig. 6); in this region two species can be differentiated, depending on the age of the polymer. The absence of some characteristic bands indicates that the contraction-reticulation of the polymeric chain is finished (connected with the older age of fossil resin); the absence of a double peak at 667 cm^{-1} in the Baltic amber spectra and the appearance of the shifted shoulder toward 1045 cm^{-1}, in the case of Romanite when compared to the Baltic shoulder, that occur at higher wavenumbers (demonstrated also by Fourier deconvolution of the spectra) provided evidence that Romanite was formed after Baltic amber (*i.e.*, it is younger), as may be observed in Fig. 6.

c. There are vibration frequency shifts determined by the degree of ethers and esters formation, correlated to the number of functional groups from the chain and showing evidence on the intramolecular bounds, which appear frequently in amber type resins with younger ages (for example the OH group, specific to Romanite, is slightly shifted due to intramolecular hydrogen bonds toward 1595 cm^{-1}, if compared to the Baltic amber where it occurs about 1640 cm^{-1}).

d. The amber has an amorphous structure, and as consequence the reproducibility of determinations is affected by the heterogeneity of the sample, the obtained spectra depending on the analysed part of the sample.

As a result of these observations, for each sample were registered two spectra, for different zones of sample, in order to observe if significant differences appear between the two, due to appearance/disappearance of certain absorption bands (specific vibration frequencies of interest). It was observed that difference between two spectra of the same sample appear only in the spectral region related to methyl, methylene, *etc.* ($-CH_3$, $-(CH_2)n-$, -CH, *etc.*) chains, namely 2962–2850 cm^{-1}, and for the specific tensile bands of CH_3 from 1375 cm^{-1}. This led to the conclusion that the presence of functional groups (which are important because they correlate with the origin and the age of the sample, and giving the sample specificity) are equally distributed. Therefore it could be considered that the analytic information supplied by FTIR is acceptable, since the wavenumbers corresponding to carboxylic chains and hydroxy-carboxylic acids at 1423-1547 cm^{-1} for Romanite and, respectively, 1300-1547 cm^{-1} for Baltic amber are preserving their region for each duplicate recorded spectrum.

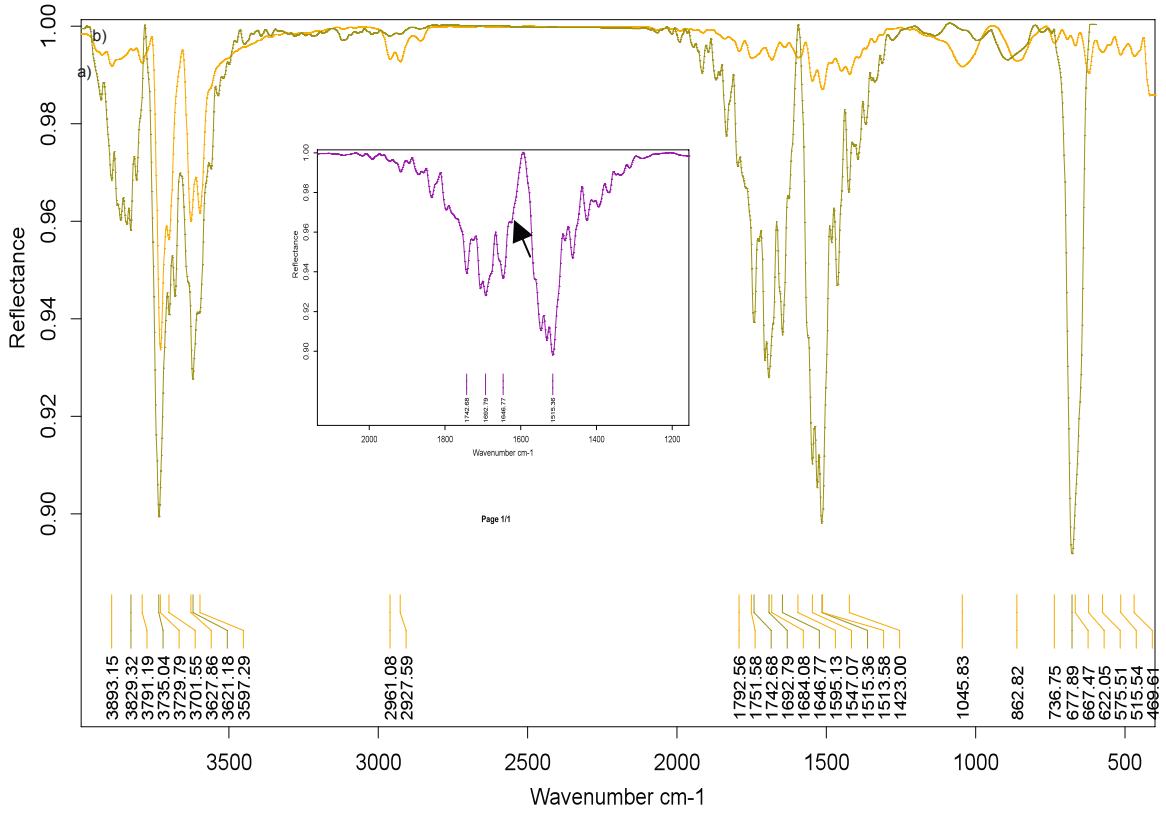

Figure 7. FTIR spectra for sample a) 321___ (and inset), and sample b) 204___ (Romanian amber samples)

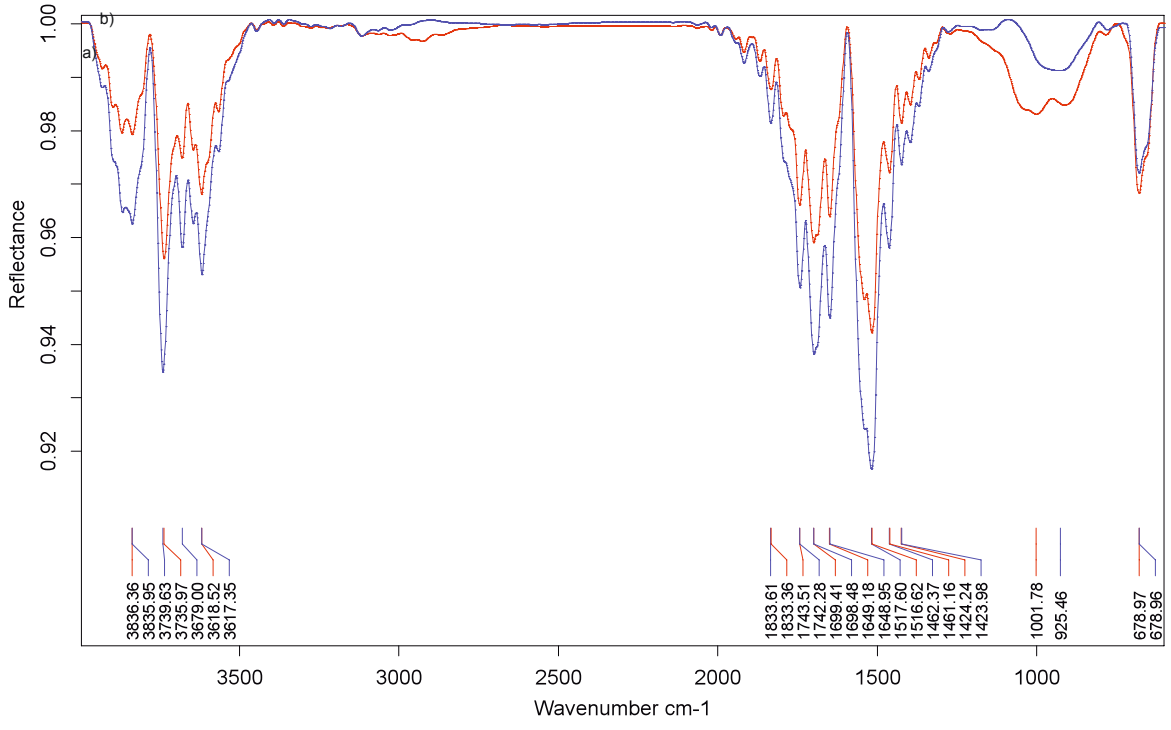

Figure 8. FTIR spectra for samples a) 341___ and b) 345___ (Baltic amber samples)

Consider two representative samples from each category, Romanite and Baltic amber, their FTIR spectra are presented in Figs. 7 and 8.

The differences appearing in the 1642-1684 cm^{-1} band of Baltic amber with respect to the 1547 cm^{-1} band of Romanite are determined by the shift of asymmetric vibration frequencies for the carboxylic type groups, as a function of the length of hydrocarbon chains. A strengthening-ageing of polymeric chains leads to shifts toward smaller wavenumbers. This can be observed also in 1046-900 cm^{-1} region, where the shifts on the smaller wavenumbers are related to the better confirmation of the Baltic origin. The significant regions for each type of amber are summarised in Table 2.

To elucidate this problem, of the age of the structures, and to confirm/deny the stated hypothesis, FTIR data should be correlated with N^{15} and C^{13} analysis.

Comparing FTIR results with LC-MS results shows that they are correlated, the sample 280 which presents an abnormality in the succinic acid determined by LC-MS, also poses a question when the FTIR data are analysed (data not shown).

4. Conclusion

FT-IR-VAR and LC-MS techniques were employed to obtain fingerprints related to the amber origin. Our experiments were applied on a wide range of samples with controlled origin, Baltic or Romanian. It was important to find either non-destructive methods or ones which could be applied on very small amounts of amber, because the final aim of our studies is to apply these methods on archaeological material. We have presented some light-microscopy images of Romanian amber, because these are practically unknown for the scientific community from our area of interest. Our preliminary results allowed us to use FTIR-VAR and LC-MS to separate Baltic amber and Romanite artefacts.

Acknowledgements

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Table 2. FTIR spectra regions of interest for amber.

Sample ID/origin	Wavenumber domain (cm^{-1})	Assign signal	Obs.
321/Colti Patarlagele -gallery	3200-3893	OH frequencies, alcoholic (phenolic free OH and bound intramolecular OH) from hydroxy-acids; observed wavenumber shifts characteristic of amorphous structures.	Identical with Romanite-Colti), differences only in intensity of specific bands
	1600 – 1684	C=C; C=O frequencies of bond vibrations; out of plane vibration of bonded C-H-C. Plane vibration of H bonds (to O). Assignment confirmed by the peaks from 620-750 cm^{-1} domain. 1600-1604 $-COO^{\cdot}$ unsaturated from carboxylic acids;	
	1617	OH group, specific to Romanite, that usually occurs as wide band at 1595 cm^{-1} (see 204) shifts and appears as shoulder toward 1617 (321), respectively 1625 (280, 383), due to H intramolecular bonds (inset in Fig. 7).	
	1423-1547	Specific for carboxylic chains and hydroxy-carboxylic acids;	
	1046-doublet	Specific peak OH & C=O, slightly shifted due to intramolecular bonds.	
	620-750	Out of plane vibrations of C=O; combined bonds with those specific to C-C=O;	
341/Kaliningrad	3200-3893	Same assignment as above, the differences appear in intensity and slight shifts, due to the different number of intramolecular bonds with respect to those of Romanite;	Specific for Baltic amber
	1600 – 1684	Same assignment as above, the differences appear in intensity and slight shifts, due to the different number of intramolecular bonds with respect to those of Romanite;	
	1300-1547	Same functional groups, carboxylic chains and hydroxy-carboxylic acids;	
	1155-1275	Baltic shoulder	
	1001/1046	Very well defined peak of OH & C=O; compared to 1046-doublet of Romanite, has different shape and is shifted toward smaller wavenumbers in Baltic amber;	
	620-750	Single peak, sharp, intense, specific for C-O, region slightly different in shape compared to Romanite;	

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