Three different nucleophilic compounds are usually used for acid-catalyzed depolymerization of proanthocyanidins, namely toluene-α-thiol (Prieur et al., 1994), phloroglucinol (Kennedy et al., 2001) and cysteamine (Torres et al., 2001). Each nucleophile has advantages and disadvantages: nucleophilic power, smell, toxicity, stability of the adducts. It would be also of interest to have different possibilities to analyse proanthocyanidins depending of raw materials. Phloroglucinol and cysteamine adducts are more polar than toluene-α-thiol ones and may be more easily separated from other polyphenols such as anthocyanins, pyrananthocyanins (Atanasova et al., 2002) and anthocyanin-flavonol adducts (Remy et al., 2000) in complex samples like wine, for example. We compared efficiency, reproducibility, accuracy of the reaction with these nucleophiles for analysis of proanthocyanidins from grape seed, grape skin, red wine polyphenol extract and finally red wine.

**Experimentation**

Depolymerization was followed by HPLC analysis on C18 column that exhibits enhanced retention of polar compounds (Atlantis dC18, Waters). LC-MS analyses in positive mode were performed on the same column to identify components of the reaction media. The stability of the reaction products was studied under different conditions and percentage of epimerization was calculated for terminal units. Proanthocyanidin composition (i.e: mean degree of polymerization (DPm), % of galloylated and trihydroxylated units, concentration) was determined using the three reagents, after isolating and calibrating each of the reaction products.


**Results**

Depolymerization with phloroglucinol and toluene-α-thiol gave higher reaction yields on isolated dimers, trimers, and tetramers (Table 1). Epimerization was very important with cysteamine, clearly present with toluene-α-thiol and may be more easily separated from other polyphenols such as anthocyanins, pyrananthocyanins (Atanasova et al., 2002) and anthocyanin-flavonol adducts (Remy et al., 2000) in complex samples like wine, for example. We compared efficiency, reproducibility, accuracy of the reaction with these nucleophiles for analysis of proanthocyanidins from grape seed, grape skin, red wine polyphenol extract and finally red wine.

**Conclusion**

Advantages and limits of the three nucleophiles used in proanthocyanidin analysis are summarized below. Controlling reaction conditions is essential to ensure accurate quantification.

Toluene-α-thiol
- excellent nucleophile
- epimerization
- low olfactory threshold 0.0005ppm
- adducts coeluted with anthocyanins

Phloroglucinol
- odorless
- low epimerization rate
- polarity of adducts
- unstable reagent

Cysteamine
- high olfactory threshold
- high epimerization rate
- lower yield
- formation of by-products

**Table 1:** Comparison on depolymerization. Epimerization= % epimerization on terminal units; new adduct= % additional adduct / terminal units; ND: not detected.

<table>
<thead>
<tr>
<th>DPM</th>
<th>%galloylated units</th>
<th>Concentration</th>
<th>%trihydroxylated units</th>
<th>Epimerization</th>
<th>New adduct</th>
<th>Recovery</th>
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<td>C</td>
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It does not reflect their views and in no way anticipates the Commission’s future policy in this area.

**References**


