Food & Flavor

Applications note

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Analyzing Free Fatty Acids

Free fatty acids are difficult to analyze by gas chromatography due to their general lack of volatility and adsorptive nature. Consequently, many laboratories involved in testing food fat content frequently analyze fatty acids in their methyl ester form. However, derivatization increases sample preparation time and cost and can add a degree of uncertainty due to the possibility of sample loss or incomplete methylation. Because of these factors, many labs prefer to analyze fatty acids in their underivatized form.

Obtaining Free Fatty Acids

One can analyze existing free fatty acids in a matrix or saponify fats to obtain them. Procedures are outlined in AOAC methods 971.11D and 938.09D[1]. Samples are extracted with solvent and saponified by heating under a reflux with an excess of dilute aqueous ethanolic alkali. After saponification, the sample is neutralized with dilute hydrochloric acid or sulfuric acid. In many cases, an aqueous solution of phosphotungstic acid is added after mixing or shaking. The sample may be centrifuged and/or filtered, and then finally diluted to an aqueous solution.

Injecting Free Fatty Acids

Higher molecular weight free fatty acids can be difficult to analyze by split/splitless injection due to discrimination of high boiling components. To minimize loss from discrimination, a direct injection technique is recommended. Direct injection will also reduce the risk of losing volatile low molecular weight fatty acids through the split vent, which improves quantitative reproducibility. Since free acids can be adsorbed, the analyst must make every effort to ensure an inert sample pathway by using properly deactivated direct injection liners and inert capillary columns. Regular preventive maintenance of the GC injection port is strongly recommended to prevent surfaces from becoming active over time.

Selecting a Column

The capillary column most widely used for volatile free fatty acids is the Stabilwax®-DA. The Stabilwax®-DA is a bonded Carbowax® column that has been specifically deactivated for acidic compounds. **Figure 1** (on the following page) shows the analysis of C_2 to C_7 fatty acids on a 15-meter, 0.53mm ID, 1.0µm Stabilwax®-DA column. Because this polar column has a strong affinity for free acids, excellent separation can be achieved even with a relatively short, 15-meter length. In addition, analysis time is reduced to only 6 minutes using the 0.53mm ID column. The unique deactivation of the Stabilwax®-DA column produces sharp peaks with minimal volatile fatty acid tailing.

For more information on molecular weight discrimination, call (800) 356-1688, ext. 5 to request a copy of our technical guide, *"Operating Hints for Split/Splitless Injectors"*.

Have Questions? Call Restek's technical service staff at:
800-356-1688, ext. 4