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### POTENTIAL OF ON-LINE LC-FTIR HYPHENATED TECNIQUE AS A RELIABLE TOOL FOR IDENTIFICATION

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LC-IR or HPLC-IR is a hyphenated technique developed from the coupling of an LC and the detection method infrared spectrometry (FTIR). While, chromatography on LC column based on partially porous particle technology, affording high peak capacity and excellent reproducibility of peak retention times/areas, is one of the most powerful separation techniques available today; FTIR is a good spectroscopic technique for the identification of organic compounds, in particular for the composition and structure of isomeric compounds, providing valuable information about local configuration of atoms in molecules.

Hyphenated technique based on HPLC-FTIR can fill a gap in identification by mass spectrometry (MS), in discriminating geometrical isomers and diastereomers and closely related molecules and can be effective whenever unambiguous identification of low-level sample constituents is required. The more successful coupling of LC and FT-IR is accomplished by solvent elimination prior to IR detection; this method involves an interface that evaporates the eluent and deposits the analytes onto a medium compatible with FT-IR detection.

The FTIR system here presented is connected to the outlet of a LC column, through a solvent removal interface, and deposits eluents as a continuous track of sample on a rotating IR transparent ZnSe disk. The built-in interferometer simultaneously captures a set of time-ordered IR spectra from the deposit track, allowing for good quality spectra to be obtained on nanogram amount of analytes. The obtained spectra is independent of the solvents used so there are no spectral restrictions on the solvents used as eluents. In addition, spectra obtained using ZnSe closely resemble conventional KBr disk transmission spectra and ATR, and thus spectral libraries and search programs may be used for identification purposes, enforcing the reliability of IR as detection technique.

In this study, HPLC-FTIR integrated system has been employed for the separation and identification of coumarins and furocoumarins isomers and the best operating condition for

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the revelation are reported. Whereas the separation of these compounds can be problematic due to their similar polarity, chemical structures and equal molecular weight, a series of experiments performed changing both chromatographic and spectroscopic parameters allowed the detection and the chemical identification through library matches.