APPLICATION OF LKB MODEL 2140 RAPID SPECTRAL DETECTOR IN HPLC ANALYSIS OF PHENOLIC COMPOUNDS

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Due to the ability of phenolic compounds to absorb UV light, absorbance spectrophotometric, detectors are commonly used in conjunction with HPLC for enhanced sensitivity in the quantitation of these plant microconstituents. Recently, rapid-scanning photodiode array detection has been applied successfully to HPLC - UV detection systems for the analysis of a number of drugs and related compounds / Fell et al.1984, Lohse et al.1984 /.
When combined with a microcomputer, continuous spectral analysis of the eluants is possible with considerable degrees of sensitivity and selectivity. In the present investigation the aplication of linear diode array detection coupled with a 16 K-bytes microcomputer was investigated as a detection system for phenolic acids extracted from rapeseed, and in standard solutions of phenolic acids.

MATERIALS AND METHODS

The phenolic acids in rapeseed flour were extracted with 80% methanol and purified by extraction with diethyl ether following hydrolysis of the phenolic acids esters with 2 M NaOH / Zadernowski et al.1983 /. HPLC analysis was conducted on a 250 mm x 4,0 mm column packed with LiChrosorb RP 18,10 µm / Knauer column / using acetonitrile - water - acetic acid / 88:10:2 V/v/v / as eluent at 120 ml/h. The optical diode - array detector / LKB model 2140 Rapid Spectral Detector, LKB Produkter AB, Bromma, Sweden /

was equipped with a deuterium lamp and 5 µl flow ceel. The detector was interfaced with a personal computer / IBM, Boca Raton, Florida /. Color displays were printed on a colorgraphics printer / Canon, Lake Success, New York /. The linear diodearray detector provided data in three dimensions - time, absorbance and wavelength. In the present study, the third dimension, absorbance, was graphed in colour gradations / red-the highest, green-the lowest, and yellow-the intermediate absorbance /.

RESULTS AND DISCUSSION

The selective absorbance of standard phenolic acids separated by HPLC is illustrated in Fig.1. The reference absorbance setting, which can be changed troughout the run, is displayed on the left vertical axis of the figure while the time lapsed is indicated on the right vertical axis. The wavelengths from 190 to 370 are displayed on the x - axis so that a cursor line placed horizontally at any point produced a corrected spectrum of the eluant at that particular time whereas a vertical cursor represented a chromatogram at that wavelength. The isograms of that UV absorbance for each phenolic acid were symmetrical around the intensity lines and separated effectively on the time scale. The isograms could be used to select an optimal wavelength for cementional twodimensional HPLC / i.e.254 nm / for purposes of identification / Fig. 1 /, but quantitation would only be obtained from the three dimensional isogram.

Analysis of the isogram and 254 nm chromatogram of the phenolic acids isolated from rapeseed flour / Fig. 2 / indicated the presence of two major compounds eluting in 2 to 7 min. The spectra of the compound with retention time of 21 min. 19 sec. was identical to the UV spectra of sinapic acid / Fig. 1 /. The UV spectra of the compound with 28 min. 31 sec., retention time displayed a spectrum that approximated that of sinapic acid / Fig. 3 / but was shifted 9 nm towards the longer wavelenghts. It appeared that this unknown compound was a derivative of sinapic acid, differing only slightly in chemical constitution.

CONCLUSIONS

The application of the linear diode-array detector in HPLC proveded simultaneously UV spectra, isograms and chromatograms of UV absorbing compounds. These data facilitated the prompt identification of unknown compounds with a " fingerprint " identification system. In particular the Rapid Spectral Detector appeared to be a powerful tool in research on phenolic compounds in rapeseed.

REFERENCES

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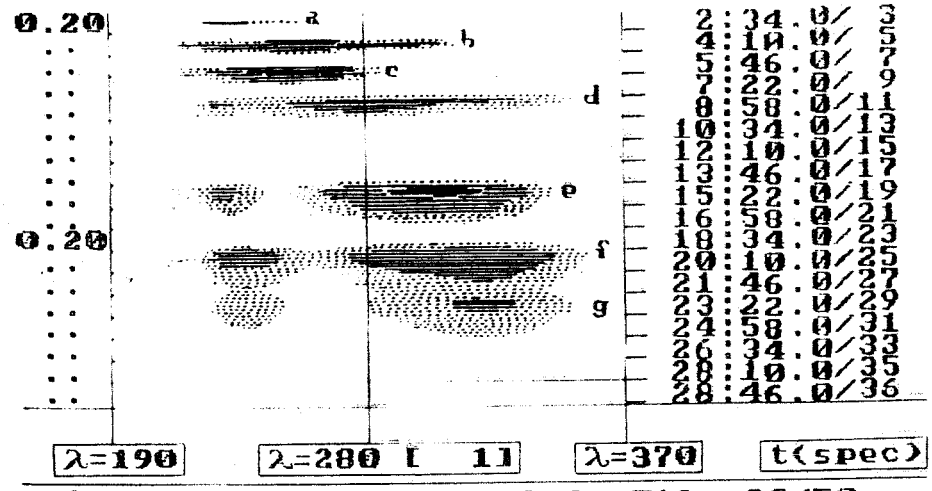
Fig. 1
Isogram and chromatogram of standards of phenolic acids



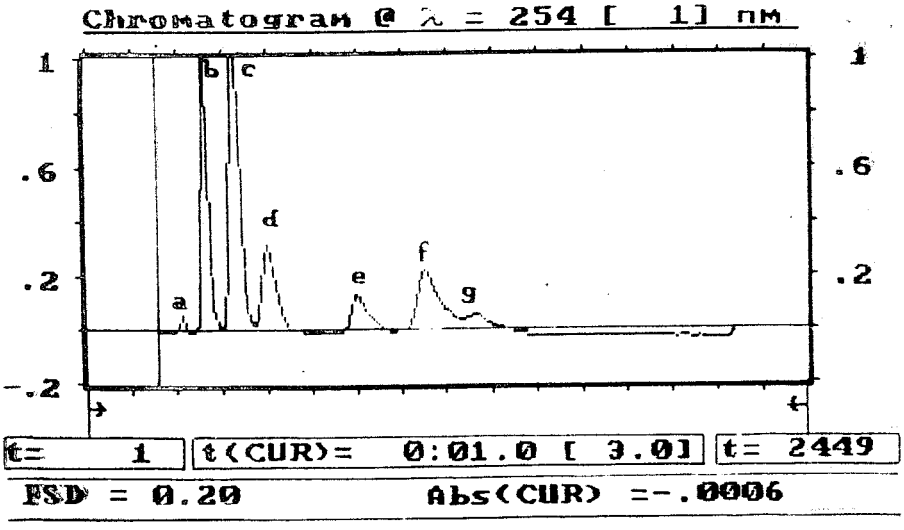
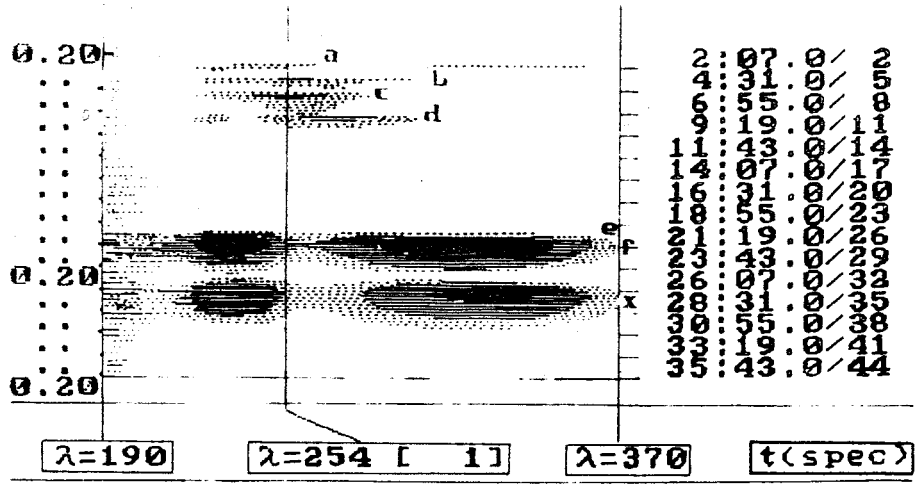


Fig. 2
Isogram and chromatogram of free phenolic acids extracted from rapeseeds



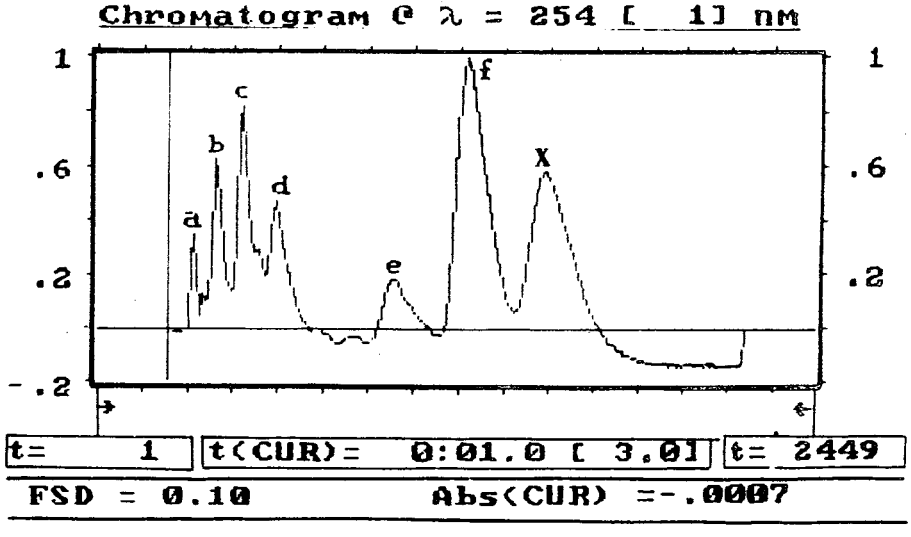


Fig. 3
UV spectra of sinapic acid standard and phenolic compounds with 21'19'' and 28'31'' retention times