Journal of Chromatography, 81 (1973) 194–195

CHROM. 6739

Note

The use of ethylenediaminetetraacetic acid in the chromatography of plant polyphenols

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(Received March 6th, 1973)

Two-dimensional chromatograms of plant polyphenols frequently show large diffuse areas of phenolic material in addition to well-defined spots. Extracts of polyphenol storage cells (purple cells)[1] of unfermented dried cacao seeds give such a diffuse area when chromatographed on paper with water or dilute acetic acid in the first direction and the top phase of butanol–acetic acid–water (4:1:5) or some similar solvent in the second direction[2]. Since it seemed possible that some of the diffuseness might be caused by oxidation on the paper, the paper was pretreated with the disodium salt of ethylenediaminetetraacetic acid (EDTA)*. Whatman 3MM paper was eluted with 1% aq. EDTA and then allowed to dry. For comparison the purple cell extract was chromatographed in one dimension on EDTA-treated and on untreated paper, using the top phase of isoamyl alcohol–ethanol–acetic acid–water (5:1:1:3) as solvent. The chromatogram on treated paper showed a striking increase in the number and clarity of spots.

The increase in clarity of the spots was not due solely to the retardation of oxidation, although oxidation was in fact retarded; on chromatograms left unsprayed for 48 h, brown spots from oxidation of the phenols showed up more strongly on the untreated paper than the treated one. However, if the paper was treated first with EDTA, dried, eluted with distilled water and dried again, the chromatograms were no better than those obtained using untreated paper. Thus, the removal of metallic ions from the paper[3] did not seem to be the primary reason for the success of EDTA treatment.

Treatment of the paper with a 0.2% solution seemed to be less effective than treatment with a 1% solution, but 0.4% and 0.5% solutions gave satisfactory results. Good two-dimensional chromatograms were easily obtained with aq. EDTA (0.4%, 0.5% or 1%) as the first solvent and a butanol solvent as the second solvent. Apart from the pigments, which streak in EDTA, the spots are clearer in the chromatograms run first in EDTA, compared to those run first in 2% acetic acid or water. With Whatman papers Nos. 1 and 2, the results are not quite as striking as with No. 3MM but there is a useful increase in definition.

The method has also been applied to thin-layer chromatography. Thin layers of cellulose powder containing 10% binder (Macherey, Nagel & Co.) were made up

* *Editor's note*: Ascorbic acid has already been used to suppress oxidation of polyphenols (F. Fish and W. R. Kirk, *J. Chromatogr.*, 36 (1968) 383).

with 0.4% aq. EDTA instead of water and the finished plates gave much better results than untreated plates. Two-dimensional chromatography with 0.4% EDTA as first solvent both on treated and untreated plates, gave better results than two-dimensional chromatography with water as first solvent. So far, column chromatography with treated cellulose powder (Solka-floc and Whatman ashless) has not been noticeably better than chromatography on untreated cellulose.

Polyphenols are commonly detected with a mixture of 1% potassium ferricyanide and 1% ferric chloride. Certain precautions are necessary to obtain good results with this reagent on chromatograms that have been treated with EDTA. The chromatogram is run through a bath of the reagent until the colours have appeared. It is then washed briefly in water and run through a bath of dilute hydrochloric acid (0.5–1.0 *N*). It is washed thoroughly with water, treated with acid and washed again and finally passed through another bath of dilute hydrochloric acid after which it is hung up to dry. During this procedure a strip of the paper must be kept dry for holding the chromatogram. If the washing and acid treatment are properly carried out, perfectly white backgrounds can be obtained, but it is important that the chromatogram be dried without further washing after acid treatment. There is, however, one disadvantage in that the acid treatment renders the chromatograms rather fragile after a few months of storage.

During the past 3 years these techniques have given good results not only with cacao polyphenols but also with polyphenols of avocado and cola.

REFERENCES

1 H. B. Brown, *Nature (London)*, 173 (1954) 492.
2 W. G. C. Forsyth, and V. C. Quesnel, *Advan. Enzymol.*, 25 (1963) 457.
3 W. L. Butler and H. W. Siegelman, *Nature (London)*, 183 (1959) 1813.