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A NEUTRAL CLEAN-UP PROCEDURE COMBINED WITH A HIGH RESOLUTION GC-MS TECHNIQUE FOR THE DETECTION OF THE 2,3,7,8-TCDD IN VARIOUS VEGETAL TISSUES

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INTRODUCTION

A study of methods described in the literature [1] reveals two extraction trends: extraction using organic solvents (i.e. hexane, benzene or hexane-acetone mixtures) and alkaline digestion followed by organic extraction. The literature states that the initial digestion of the sample makes possible saponification of the fats and the disaggregation of the organic matrix and the possibly adsorbed TCDD should be more easily extracted. So a variety of vegetal tissues have been submitted by us to a hot digestion in an aqueous medium with KOH at 10%. This resulted in a very suitable procedure for vegetal tissues containing a high percentage of water, but it is unsuitable for application to tissues with a high content of starch (i.e. potatoes, wheat, beans, rice, grains of maize etc.), because heating in an alkaline medium produces irreversible gels. On the contary, these samples are hydrolyzed in an aqueous medium with HCl at 5% and heated for 2 hours. The acid digestion is unsuitable for the vegetal tissues that do not contain starch,however, because the organic matrix cannot be disaggregated.

Alkaline digestion can prove difficult with other vegetal tissues(i.e. maize and bean plants) which sometimes form into an emulsion. Therefore alternative extraction procedures have been investigated, possibly suitable for any type of vegetal tissues. It was found that an extraction performed first with a polar solvent (methanol) and then with a non-polar one (methylene chloride) was succesful with all the species of vegetable tissues thus far tested.

METHOD

The complete method is given schematically in Figure 1. It should be noted that further improvements to the purification and separation steps are being considered in order to improve the quality of the extracts and, therefore,

to decrease the sensitivity limit.

The following comments can be made on the various analysis steps:

- Spiking (2): a double spiking has been used because with a medium mass-spectrometric resolution many environmental contaminants can interfere with fragments to the masses chosen for the native TCDD and the internal standards. The correct isotope ratio of molecular ions of the native TCDD, and the intensity ratio for the molecular ions of the labelled TCDD being equal to that expected, give the certainty of quantitative identification of 2,3,7,8 -TCDD.
- Extraction (3): a colourful mixture of products is obtained, with the majority of polar and acid compounds in the hydroalchoolic phase. From 50 grams of fresh vegetal tissues 5÷7 g of residual matrix and about 1.5 g of total organic extract residues are obtained. 1.0 g of this latter residue comes from the hydroalchoolic solution and about 0.5 g from dichloromethane. The alkaline digestion produces a less abundant residue (about 0.5 g) due to the saponification of most of the fats which remain in the aqueous phase.
- Silica-gel column (4): the dimensions of this column have been specially chosen to adsorb the polar compounds on its upper part, leading in turn to an improvement in chromatographic separation. Benzene was selected as eluting solvent because of its ability to solubilize products and because of a distinct separation between polar and non-polar products as clearly shown by TLC plates. Columns having a smaller diameter have sometimes shown obstructions with losses of TCDD.
- Multilayer column (5): the elution of this column with petroleum ether introduces an alternative chromatographic separation on silica gel based on the different polarities of the solvents. For example the silica-gel column (4) loaded with extracts of green tissues elutes some orange-yellow products (carotene, xanthophyll etc.) which ramain attached to the silica-gel of the multilayer-column (5) when petroleum ether is used. The efficacy of this "double" chromatographic separation is proved by the slight darkening of the Celite/H_2SO_4 mixture placed below the silica-gel of the multilayer column. This shows that only a few oxidable compounds are still present. The size of our column differs from that described in the literature [2]. Since the effectiveness of the purification increases with the contact time [3] between the solution of the sample and the Celite/H_2SO_4 mixture, only the quantity of the Celite has been increased. Furthermore

the dilution of the eluate of column (4) with the petroleum ether prevents both the quick oxidation of the products and carbonization with possible incorporation of TCDD.

- Florisil and alumina columns (6÷8): specific tests carried out on the florisil column (6) have confirmed that the size specified in the literature [2] is suitable to our method. However taking into consideration that high quantities of waxes, resins, lipids and fatty substances might be present in many vegetal tissues, it is advisable to proceed through a second similar chromatographic step, i.e. use of a microcolumn packed with neutral alumina and florisil (7). The final purification is obtained with a microcolumn packed with basic alumina (8).
- GC-MS measurements (9): gas-chromatography with OV-17 and/or Silar 10C capillary columns and on-column injection technique is used. Simultaneous mass spectrometric measurement (RP $\geqslant$ 10,000) are performed at masses 320 and 322 for native TCDD and the mass corresponding to the most intense molecular ion of added labelled TCDD. Greater specificity derives from the measurement of a third ion for the native TCDD (the molecular ion at m/z 324 or the fragment ion at 257) and from the measurement of the most intense molecular ion of a second differently labelled TCDD.

  Systematic analyses are performed on control samples free of 2,3,7,8-TCDD (blanks) treated in parallel with the samples examined.

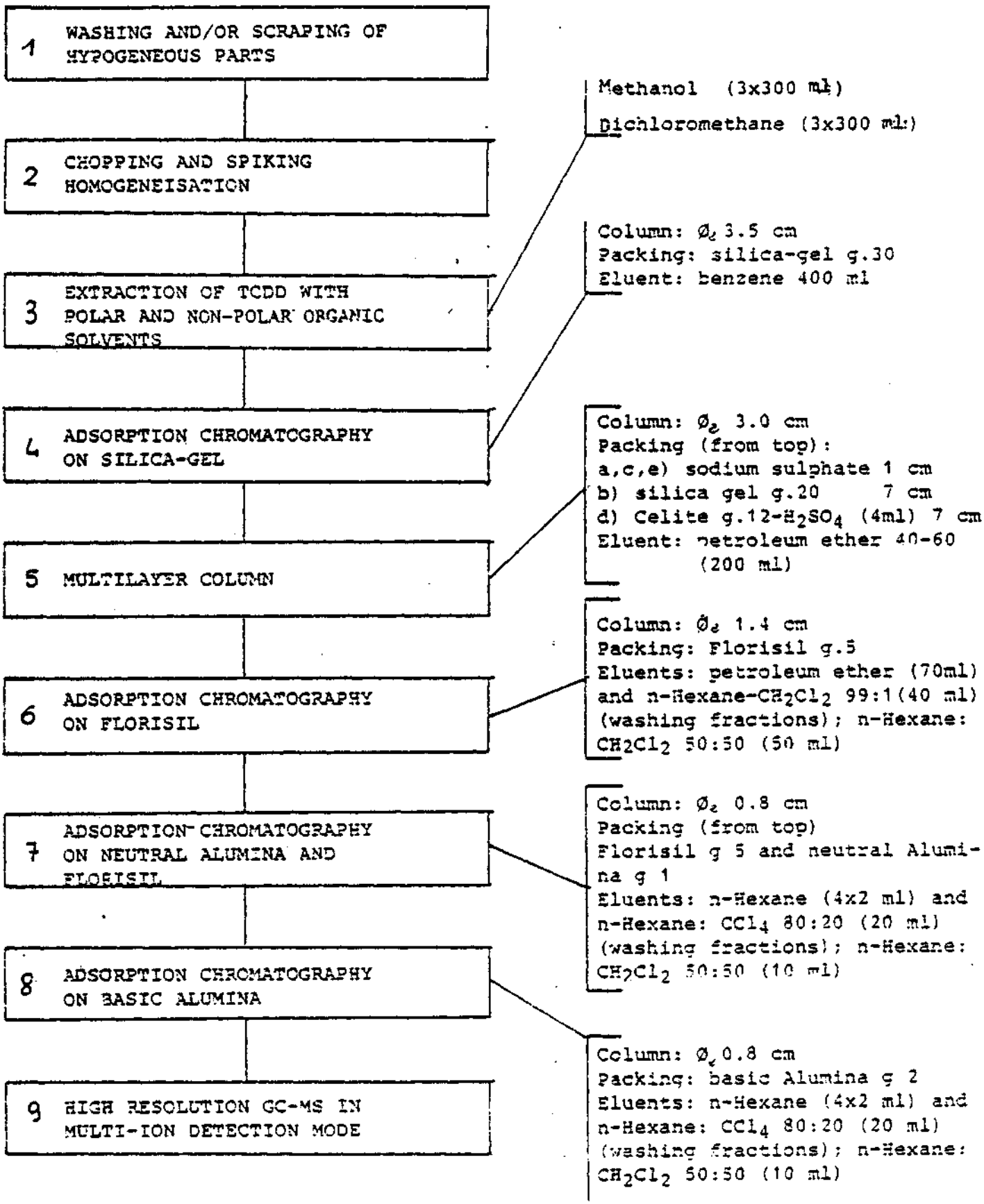
RESULTS

The step by step recoveries and the sensitivity limit have been evaluated experimentally.

.Step by step recoveries: 10 ng of 14-C-TCDD have been added to 50 g of fresh vegetal tissues. At about 200 ppt we estimated a total recovery of 80±5% which remains approximately constant throughout all the purification and separation steps. Table 1 shows the recoveries so far obtained for various vegetal tissues.

.Sensitivity limit: 1 ng of native TCDD and 5 ng of both 37Cl-TCDD and 13C-TCDD have been adde to 50g of fresh vegetal tissues (carrots and maize) free of 2,3,7,8-TCDD. Repetitive test have shown a detection limit of 5÷10 p t.

FIGURE 1



-ALL SOLVENTS ARE FOR PESTICIDE ANALYSES

TABLE 1. Step by step recoveries in various vegetal tissues

STEPS / VEGETALS	a	b	c	d
CARROT	77	79	81	79
BEAN PLANT	83	79	80	82
MAIZE PLANT	80	77	84	81
WHEAT	-	73	70	71
POTATO	78	76	75	75

a = steps 1÷5
b = steps 1÷6
c = steps 1÷7
d = complete analysis

CONCLUSIONS

The clean-up method proposed allows quantitative measurements of the 2,3,7,8-TCDD present at a level of 25 ppt in vegetal tissue samples of any origin. Further developments are under consideration in order to improve the quality of the extracts and to perform measurements that are not affected by interfering substances at about 1 ppt level.

BIBLIOGRAPHY

1) S.Facchetti and A. Leoni, Applications of Mass Spectrometry to Trace Analysis, ISPRA, Italy, 29 September-3 October 1980, S. Facchetti (Ed.)-Elsevier Scientific Publishing Company, Amsterdam

2) A. Cavallaro, G. Bartolozzi, D. Carreri, A. Gorni, L. Luciani and G. Villa, Private communication (1979)

3) H.K. Wipf, E. Homberger, N. Neuner, U.B. Ranalder, W. Vetter and J.P. Vuilleumier, Private Communication (1978)