International Journal of Pharmaceutics, 48 (1988) 119–124
Elsevier

IJP 01618

Stabilization of tinctures with cyclodextrins: a tincture from the fruits of *Oenanthe aquatica* L.

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(Received 11 January 1988)
(Modified version 29 April 1988)
(Accepted 6 May 1988)

Key words: Tincture; Cyclodextrin; Stabilization; *Oenanthe aquatica* L.; Polyacetylene; Terpene

Summary

An original system of stabilization of the active principles of tinctures by means of cyclodextrins (α, β, γ-CyD) is proposed. In the particular case of a tincture from the fruits of *Oenanthe aquatica* L. the microinclusion of the components was found to be incomplete in all 3 cyclodextrins. However, β-CyD was the most efficient since a solid form was derived from it in which the most unstable active principles, polyacetylenes and terpenes, are included in the cyclic olygosaccharide and the others, lignans and steroids, are present as a physical mixture. GC checks confirmed the full correspondence of the components of this new form to those of the tincture from which it derives. The advantages of its use lie in the stabilization of the most unstable microincluded active principles within it, which was verified by means of artificial ageing studies, and in the implication that simple and complex solid dosage forms may be more simply prepared.

Introduction

One of the main requirements for the use of tinctures is the stability of all their components, this being the essential condition for homogeneity and for certainty of therapeutic response.

The problem of stability is rendered more complex by the copresence of molecules belonging to classes having very different chemical features. Faced with such a large variety of substances, stabilization often becomes difficult to achieve, and thus a generalized procedure is adopted, with the suggestion that the tincture be kept in a cool, dry, dark environment. Alternatively, dry extracts obtained by spray-drying may be used, but this operation, which requires a very special technology and apparatus, must be carried out on an industrial level and may discriminate in favour of non-volatile active principles against volatile ones; it does not, moreover, guarantee complete stabilization of the most unstable molecules during subsequent storage.

The aim of the present study is to evaluate the use of cyclodextrins for the stabilization of tinctures whose most unstable and/or volatile active principles can be microincluded in these cyclic oligosaccharides.

Cyclodextrins are made up of either 6, 7 or 8 units of glucose and are indicated by α, β and γ, respectively. They have the property of forming

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inclusion complexes with various molecules which adapt themselves either wholly or partially to their cavities (0.5–0.8 nm; Saenger, 1980).

Inclusion complexes improve the stability of active principles against oxidation, polymerization and alterations due to light, heat and storage time, and improve the bioavailability of the "guest" molecule due to an increase in hydrosolubility and absorption (Duchêne et al., 1984; Duchêne et al., 1985).

To assess the validity of the microinclusion by cyclodextrins we chose a tincture made from the fruits of *Oenanthe aquatica* L. This is used in treating dyspepsia, bronchitis and asthma (Penso, 1983). Its composition is very singular, because besides terpenes and sterols, polyacetylene compounds and lignans are also present (Vincieri et al., 1976, 1985, 1986, 1988; Coran et al., 1987).

Terpenes and, even more, polyacetylenes are volatile and unstable compounds and subject to isomerization and polymerization.

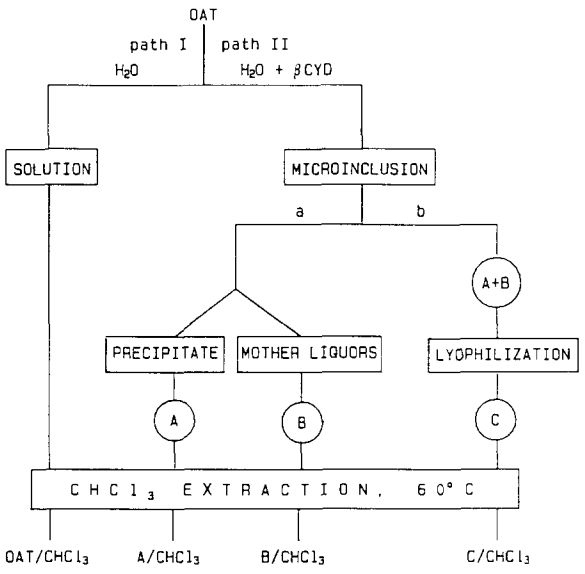
The β-cyclodextrin is the one which is most capable of forming microinclusion with substances whose molecular weight is in the range of 50–250 Da, and is thus seen to be suitable for stabilizing the above-mentioned active principles.

Materials and Methods

Materials

The *Oenanthe aquatica* L. tincture (OAT) was obtained according to Vincieri et al. (1988), and kept at −20 °C, away from light and under N_2. The cyclodextrins (α-, β-, γ-CyD) were purchased from Fluka and used as they were. The water was deionized and doubly distilled.

Gas chromatography was performed on a Perkin Elmer 8320 instrument with FID detector, fitted with a programmed temperature vaporizer (PTV) and built-in data handling facility. Oven temperature went from 50 to 100 °C at 5 °C/min, and from 100 to 300 °C at 10 °C/min and was kept at 300 °C for 10 min; detector temperature 330 °C; cold injection and vaporization at 320 °C, column SGE 12QC2/BP1 0.25.



Scheme 1

Thermal analyses were performed on a Perkin Elmer DSC-4 calorimeter equipped with a 3600 Data Station; scanning speed 10 °C/min.

Lyophilization was performed on a Leybold Liovac GT 2 laboratory lyophilizer.

Methods

The whole preparation procedure is reported in scheme I.

Sample preparation

2.5 ml OAT were placed under stirring with 50 ml aqueous solution saturated with β-CyD at 25 °C. After 24 h (path IIa), the precipitate (A) was recovered from the mother liquors (B) and dried under vacuum over $CaCl_2$. Alternatively (path IIb), after the formation of the precipitate, the whole (A + B) was lyophilized to obtain C.

A blank solution was also prepared in the same way working on 2.5 ml of OAT dissolved in 50 ml H_2O which was shaken at 25 °C for 24 h (path I).

The physical mixture of OAT and β-CyD was obtained by careful mixing of 0.5 ml of OAT and 180 mg of β-CyD and dried under vacuum over $CaCl_2$.

Samples of OAT (2.5 ml) and C (1 g) were submitted to artificial ageing by keeping them in

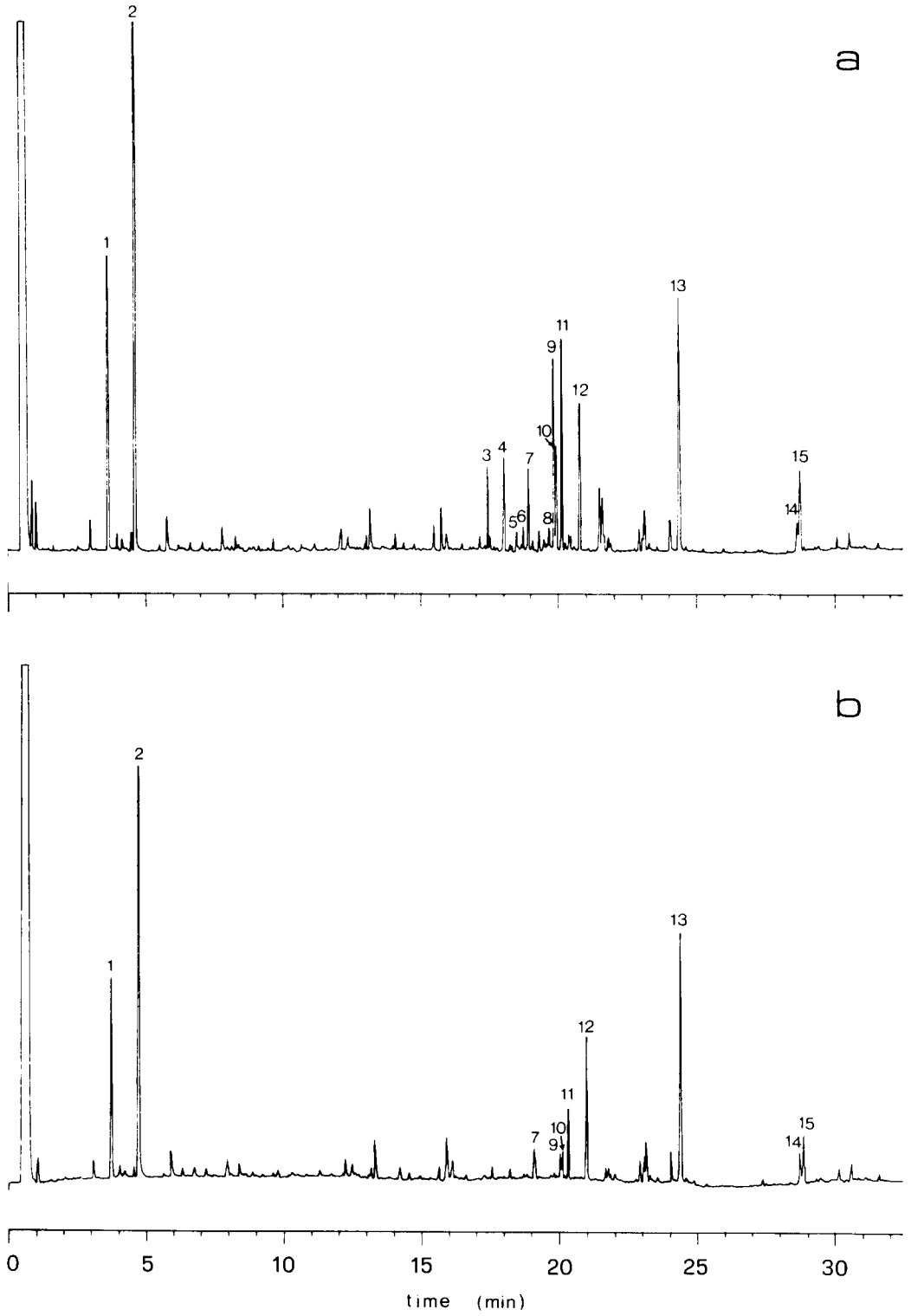


Fig. 1. Gaschromatograms of OAT/$CHCl_3$ (identical to those of C/$CHCl_3$ and C1w/$CHCl_3$, C2w/$CHCl_3$, C3w/$CHCl_3$, C4w/$CHCl_3$) (a) and OAT1w/$CHCl_3$ (identical to those of OAT2w/$CHCl_3$, OAT3w/$CHCl_3$, OAT4w/$CHCl_3$) (b) compared to show the protection exerted by microinclusion-adsorption process. 0–17 min, terpene range; 17–22 min, polyacetylene range; 22–25 min, alkane range; 25–35 min, lignan and steroid range. For individual components identification see text.

an environment which was thermostatted at 50 °C for 4 weeks, and monitoring them periodically after 1, 2, 3, 4 weeks.

Analysis of components

The analysis of the components present in A and C before and after ageing and in the mother liquors was performed in comparison with a blank using a combination of TLC and GC and working on $CHCl_3$ extracts of each of the above samples. The components selected to evaluate the whole process were (Fig. 1):

1 β-pinene
2 limonene
3 2-*trans*-9-*cis*-2,9-pentadecadiene-4,6-diyne
4 2-*trans*-8-*cis*-10-*trans*-2,8,10-pentadecatriene-4,6-diyne
5 2-*trans*-8-*cis*-10-*cis*-2,8,1-pentadecatriene-4,6-diyne
6 2-*trans*-8-*trans*-10-*cis*-2,8,10-pentadecatriene-4,6-diyne
7 2-*trans*-8-*trans*-10-*trans*-2,8,10-pentadecatriene-4,6-diyne
8 2-*trans*-8-*trans*-2,8-pentadecadiene-4,6-diyne-10-one
9 2-*trans*-8-*cis*-10-*trans*-2,8,10-pentadecatriene-4,6-diyne-12-ol
10 2-*trans*-8-*trans*-2,8-pentadecadiene-4,6-diyne-12-one
11 2-*trans*-8-*trans*-2,8-pentadecadiene-4,6-diyne-10-ol
12 2-*trans*-8-*trans*-10-*trans*-2,8,10-pentadecatriene-4,6-diyne-12-ol
13 *n*-nonacosane
14 *trans*-matairesinol(2,3-bis(3-methoxy-4-hydroxybenzyl)-4-butanolide)
15 *trans*-dimethylmatairesinol(2,3-bis(3,4-dimethoxybenzyl)-4-butanolide)

The extraction procedure consists of dissolving the solid samples in 50 ml of H_2O at 60 °C, the solution being shaken for 10 min with $CHCl_3$ (3 × 30 ml) at the same temperature. After cooling, the organic phase is separated, dried over anhydrous Na_2SO_4 and concentrated under vacuum in a small volume ($A/CHCl_3$ for A and $C/CHCl_3$ for C).

The mother liquors (B) are extracted in a similar way ($B/CHCl_3$).

The blank ($OAT/CHCl_3$) is also obtained by $CHCl_3$ extraction of path I solution.

The OAT and C samples which have been artificially aged for 1, 2, 3, 4 weeks, and extracted as above, are indicated as $OAT1w/CHCl_3$, $OAT2w/CHCl_3$, $OAT3w/CHCl_3$, $OAT4w/CHCl_3$ and $C1w/CHCl_3$, $C2w/CHCl_3$, $C3w/CHCl_3$, $C4w/CHCl_3$, respectively.

TLC was used in a routine way for a rapid assessment of the optimum extraction conditions, using Merck HPTLC silica gel 60 F_{254} 10 × 10 cm plates (art. 5628), $CHCl_3$ as eluent. 1 ml conc. H_2SO_4 added to a solution of 0.5 ml anisaldehyde in 50 ml CH_3COOH was used as spray reagent. After spraying, the plates were heated at 100–105 °C until the spots attained maximum colour intensity.

The gas chromatographic qualitative analysis was done by comparing the peak retention time of the various samples with those of $OAT/CHCl_3$.

The quantitative analysis was based on measurement of peak areas using the peak area of *n*-nonacosane, a hydrocarbon present in OAT, as internal standard. The same component served as internal reference and for assessing stability. In fact pure *n*-nonacosane, alone and with β-CyD in 70% EtOH solution, was unaffected by the artificial ageing procedures.

DSC were also performed on A and C samples and on the physical mixture of OAT and β-CyD.

Results and Discussion

First of all we assessed the results obtained by treating OAT with the 3 cyclodextrins (α-, β-, γ-CyD), following path IIa, i.e. comparing the gas chromatographic traces of $A/CHCl_3$ and $B/CHCl_3$ with those of $OAT/CHCl_3$.

The microinclusion of OAT was found to be incomplete in all 3 CyDs. However, of the 3, the β-CyD was the most efficient, managing to microinclude the greatest number of components, specifically all the terpenes and all the polyacetylenes, while the steroids and lignans remained in the mother liquors.

The validity of the test analytical method was proved by the full correspondence of the contents

of $A/CHCl_3$ to those of $B/CHCl_3$ as compared to the $OAT/CHCl_3$.

The obtaining of A with path IIa is thus a valid method for microincluding the most unstable compounds of OAT, i.e. the terpenes and polyacetylenes. Confirmation that precipitate A was in fact a microinclusion was given by the DSC analysis. In fact, in the range 50–250 °C the thermogram showed no endothermic peaks due to the reduction in volatility of the included compounds (Uekama et al., 1983), while the physical mixture of β-CyD and OAT showed 3 endothermic peaks at 114, 129, and 213 °C, as well as that of the residual solvent around 100 °C.

No polymerization or isomerization took place in A since, as mentioned above, the contents of $A/CHCl_3$ with those of $B/CHCl_3$ fully corresponded to $OAT/CHCl_3$.

In any case the inclusion product thus obtained cannot be considered a substitute for OAT in that it lacks the steroids and lignans. To get over this we assessed path IIb, still using β-CyD. With this path an overall lyophilization is performed after microinclusion and a product C is obtained; in this the non-microincluded components are adsorbed on to the microinclusion itself.

Product C was first assessed with DSC. The thermogram showed two endothermic peaks in the range 50 °–250 °C, one at 114 °C and the other at 214 °C, which are significant for the copresence of substances in a physical mixture with β-CyD; this result differed from the one obtained for the pure microinclusion A.

The subsequent GC comparison of $C/CHCl_3$ and $OAT/CHCl_3$ confirmed that all the components present in the blank were also present in C and that their relative quantities were wholly comparable.

Product C obtained with β-CyD following path IIb is thus seen to contain all the components of OAT, partly microincluded and partly adsorbed, and it may therefore be taken to represent a useful alternative to it. The advantages of its use over OAT lie mainly in the stabilization of the terpenes and polyacetylenes.

This was verified by means of a GC assessment of the effect of the artificial ageing on the components present in OAT and C. The GC of $OAT1w/CHCl_3$ (Fig. 1b) differs from $OAT/CHCl_3$ (Fig. 1a) because of both a slight decrease in terpenes and lignan **15**, and marked decrease in polyacetylenes. Under our experimental conditions in $OAT1w/CHCl_3$ there is in fact a drastic decrease in all polyacetylenes except alcohol **11** and hydrocarbon **7**, which decrease by about 30%, and alcohol **12**, which remains unaltered. It is interesting to note how all 3 of these polyacetylenes show all-*trans* isomery, even if no conclusive explanation of this behaviour can be offered.

One might hypothesize that all-*trans* polyacetylenes are in general more stable than corresponding *cis* isomers and/or that the latter partly isomerize into the *trans* form during ageing. Further work is required to shed light on this.

During the first week of ageing no change in hydrocarbon **13** or in the remaining steroids or lignans **14** and **15** was seen. The variations which took place after a week's ageing stayed practically constant in the subsequent 3 weeks as the GC of $OAT2w/CHCl_3$, $OAT3w/CHCl_3$, $OAT4w/CHCl_3$ demonstrate. On the other hand, the ageing effects on C are very different. The microinclusion-adsorption process of the OAT components causes them to be subsequently protected against ageing connected phenomena.

The GC traces of $C1w/CHCl_3$, $C2w/CHCl_3$, $C3w/CHCl_3$, $C4w/CHCl_3$ are in fact identical to one another, to $C/CHCl_3$ and to $OAT/CHCl_3$. One is moreover led to expect an increase with C in the bioavailability of the most lipophilic active principles present in it; research is currently in progress on this topic.

As regards pharmaceutical formulations it may be claimed, in view of these results, that the application of the combination microinclusion–lyophilization seems more suitable than use of the tincture as it stands when simple solid dosage forms, like hard gelatine capsules, or more complex ones, like tablets, have to be prepared.

Acknowledgements

This research was supported by grants from Italian Ministero della Pubblica Istruzione. Thanks

are due to Dr. R. Hoffmann and Mrs. S. Gallori for their technical assistance.

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