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Food Chemistry 93 (2005) 349–353

Food Chemistry

www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

Molecularly imprinted solid phase extraction for detection of sudan I in food matrices

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Received 6 July 2004; received in revised form 10 November 2004; accepted 16 November 2004

Abstract

Sudan I is a synthetic azoic dye employed as an additive in foods, and in particularly in those containing chilli powders, because of his intense red–orange colour. Now European Community does not allow "Sudan I" as an additive in foods because of its demonstrated cancerogenity.

Several methods were developed to detect the presence of this compound (HPLC, GC/MS). However when "Sudan I" is mixed just in traces in foods, very expensive instruments are necessary (LC/MS/MS) to reveal it.

This study describes the synthesis of a molecularly imprinted polymers (MIPs) for solid phase extraction (MISPE) using Sudan I as template. This procedure allows to concentrate this compound in order to make it detectable by HPLC. Furthermore we investigated the ability of these MISPE cartridges to absorb selectively Sudan I from food matrices. Considerable differences in the interaction with "Sudan I" were observed when MIPs were used as stationary phase in SPE compared with the non imprinted polymers (NIPs).

Keywords: Molecularly imprinted polymer; Solid phase extraction; Sudan I; Red chilli peppers

1. Introduction

The aim of this work was the development of Sudan I (Fig. 1) molecularly imprinted polymer useful for solid phase extraction (MISPE) and the evaluation of their performance as potential analytical strategy to monitor the illegal use of this compound. Sudan I (1-phenylazo-2-naphthol, CAS 842-07-09) is a synthetic azoic dye, used in many fields such as textile industry, for colouring solvent and floor polishes. For many years it has been widely employed as an additive in foods, particularly in those containing chilli powders, because of his intense red–orange colour (Capitàn, Capitàn-Vallvey, Fernàdez, De Orbe, & Avidad, 1996); it was also found in a number of relishes, chutneys and seasonings (http://www.foodstandards.gov.uk/safereating/sudani).

Now European Community does not allow Sudan I as additive in a food because of its cancerogenity was demonstrated (Commission Decision (E.C.) n.460/2003 of 20 June, 2003). Several methods were developed to detect the presence of this compound (HPLC, GC/MS) (Chen, Mou, Hou, Riviello, & Ni, 1998; http://www.osha.gov/dts/chemicalsampling/data/CH_268420.html). However when "Sudan I" is mixed just in traces in foods, very expensive instruments are necessary (LC/MS/MS) to reveal it (Tateo & Bononi, 2004). We propose a new methodology to concentrate, purify and then detect "Sudan I" with a known instrument (HPLC). This study describes the synthesis of a molecularly

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doi:10.1016/j.foodchem.2004.11.014

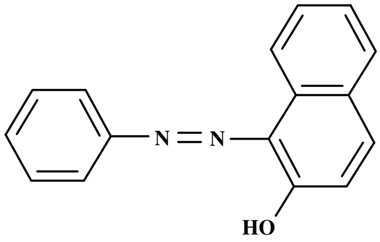

Fig. 1. Sudan I.

imprinted polymers for solid phase extraction (MISPE) using Sudan I as template. Molecular imprinting is an efficient method to introduce specific molecular recognition sites into a polymeric matrix (Caro et al., 2002). The applicability of this technique has led to numerous reports; MIPs were used as stationary phases in chromatographic separation and chiral separation (Haupt, 2001). MIPs were used in immunoassay-like analyses, as synthetic enzymes, as drug delivery systems (DDS) (Puoci et al., 2004) and in agrofood industry particularly in areas such as analysis, extraction, and preconcentration of components (Molinelli, Weiss, & Mizaikoff, 2002; Ramström, Skudar, Haines, Patel, & Brüggemann, 2001). The non-covalent approach technique, used to produce MIPs, involves arranging functional monomers around a templating ligand. This ligand is a selected target substance and it should form a prepolymerization complex with the monomer by non-covalent interactions such as hydrogen bonding, ionic or hydrophobic interactions. The formed complex is subsequently radically copolymerized with a suitable crosslinker. After copolymerization, template is removed to obtain free binding sites available to rebind selectively the original template (Puoci et al., 2004). In this work we synthesized two different kinds of imprinted polymers using, respectively, methacrylic acid (MAA) and 4-vinylpyridine (4-Vp) as functional monomer and ethylene glycol dimthacrylate (EGDMA) as crosslinker. The best results were achieved using polymers based on 4-vinylpyridine as stationary phase to pack MISPE cartridges. We investigated the ability of these MISPE cartridges to absorb selectively Sudan I from food matrices. This procedure allows to concentrate this compound. After its elution from MISPE cartridges it is possible to analyze the concentrated eluate by HPLC.

2. Materials and methods

2.1. Materials

Ethylene glycol dimthacrylate (EGDMA), methacrylic acid (MAA), 4-vinylpyridine and 2,2′-azobis-(isobutyronitrile) (AIBN) were obtained from Aldrich. All solvents were reagent grade or HPLC-grade and used without further purification and they were provided by Fluka Chemie. “Sudan I” was purchased from Aldrich.

2.2. Apparatus

HPLC was performed using a Jasco Model (Tokyo, Japan).

2.3. Synthesis of Sudan I molecularly imprinted polymer

The MIP stationary phase was prepared by bulk polymerization (Caro et al., 2002). Methacrylic acid and 4-vinylpyridine were, respectively, used as the functional monomer to prepare the MIP by the non-covalent imprinting method. Briefly, 2.5 mmol of template Sudan I, 8 mmol of methacrylic acid, 40.4 mmol of EGDMA and 103 mg of AIBN were dissolved in 4 ml of chloroform in a thick-walled glass tube. The resultant solution was purged with nitrogen and sonicated for 10 min. The mixture was, then, incubated under a nitrogen atmosphere at 68 °C for 24 h. When 4-vinylpyridine was used as the functional monomer, the recipe was: Sudan I (2.5 mmol), 4-vinylpyridine (8 mmol), EGDMA (40.4 mmol) and AIBN (103 mg); all were dissolved in 5 ml of chloroform. EGDMA was used as the crosslinking monomer and AIBN as the free radical initiator. The resultant bulk rigid polymer was crushed, grounded into powder and sieved through a 63 nm stainless steel sieve. The sieved MIP material was collected and the very fine powder, suspended in the supernatant solution (acetone), was discarded. The resultant MIP material was soxhlet extracted with 200 ml of acetic acid:methanol (1:1) mixture for at least 48 h followed by 200 ml of methanol for another 48 h. The extracted MIP material was dried in an oven at 60 °C overnight. The washed MIP material was checked to be free of “Sudan I” and any other compound by HPLC analysis. Blank polymers (to act as control) were prepared under the same conditions except that no template was used.

2.4. Preparation of MISPE columns

The 500 mg amount of dry particles of polymer was packed into a 6.0 ml polypropylene SPE column. The column was attached with a stop cock and a reservoir at the bottom end and the top end, respectively. The polymer was rinsed with methanol and then with chloroform.

2.5. MISPE procedure

To calculate MISPE cartridges loading capacity and selectivity, “Sudan I” was dissolved in chloroform to final concentrations of 10, 50, 100 μg/ml, respectively. A dry MISPE column was rinsed with 10 ml of chloroform followed by loading of 1–10 ml of standard solutions. The total amount of the analyte loaded on the column was always 100 μg. After column drying, 5 ml of methanol (elution solvent) were applied to perform the complete extraction of "Sudan I".

2.6. Molecularly imprinted solid phase extraction from a food matrix

We decided to calculate the performance of MISPE cartridges, compared to the blank one, using a red chili powder spiked with Sudan I (10 μg/g of red chilli powder). Briefly, 2 g of the red chili powder were extracted with 30 ml of chloroform. The obtained solution was filtered and then used to load (12 ml) the MISPE column. The column was then washed with 0.3 ml of dichloromethane and finally 4 ml methanol was used to elute. The elution fraction was analyzed by HPLC.

2.7. HPLC analysis

The liquid chromatography consisted of a Jasco BIP-I pump and the Jasco UVDEC-100-V detector set at 254 nm. A 250 × 4 mm C-18 Hibar® column, particle size 10 μm (Merck, Darmstadt, Germany) was employed. The mobile phase was methanol/acetonitrile, (4/6, v/v) and the flow rate was 0.5 ml/min.

3. Results and discussion

We synthesized two kinds of bulk molecularly imprinted polymers: MAA-MIPs polymer, using methacrylic acid as functional monomer and 4VP-MIPs polymer, using 4-vinylpyridine. All the prepared MIPs were crosslinked polymers and the recognition sites for "Sudan I" were successfully introduced. Different extractions and washing solvents were used to estimate the imprinting effect of these MISPE cartridges (Table 1).

The best results were obtained washing the MISPE cartridges with dichloromethane (0.3 ml) and using methanol to elute. It is possible to note a different behaviour of the two polymers: MAA-MIPs does not show a good imprinting effect, instead the 4VP-MIPs has a better one (Figs. 2 and 3).

Probably these different effects depend on the strength interactions between hydroxilic group of "Sudan I" and the functional group of the monomer used.

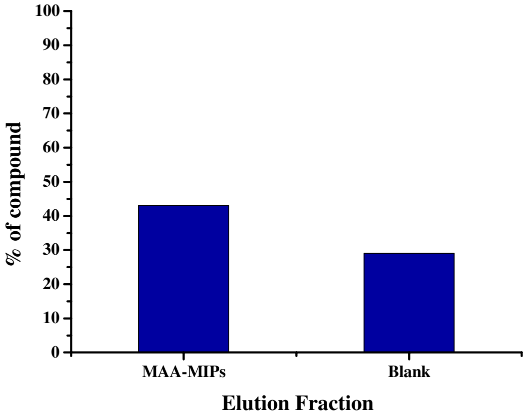

Fig. 2. Comparison of recoveries of Sudan I in elution fraction related to MAA-MIPs column and blank column after solid phase extraction of standard solution of sudan I.

Table 1
Elution profile of 100 μg Sudan I on 500 mg MISPE and Blank columns (loading solution in chloroform)

Washing solvent (0.5 ml)	Elution solvent (4 ml)	% Collected			
		MAA-MIPs	4VP-MIPs	Blank MAA	Blank 4-VP
Methanol	–	58[a]	87[a]	45[a]	46[a]
Methanol	Methanol	n.d.	n.d.	n.d.	n.d
Methanol	Methanol with 1% of acetic acid	n.d.	n.d.	n.d.	n.d.
Acetonitrile	–	27[a]	18[a]	32[a]	31[a]
Acetonitrile	Acetonitrile with 5% of acetic acid	30[b]	70[b]	12[b]	12[b]
Acetonitrile	Methanol	31[b]	69[b]	13[b]	12[b]
Acetonitrile	Methanol with 1% of acetic acid	30[b]	70[b]	12[b]	13[b]
Chloroform	–	17[a]	11[a]	24[a]	22[a]
Chloroform	Acetonitrile with 5% of acetic acid	42[b]	75[b]	20[b]	22[b]
Chloroform	Methanol	43[b]	76[b]	21[b]	22[b]
Chloroform	Methanol with 1% of acetic acid	41[b]	77[b]	21[b]	23[b]
Dichloromethane	–	17[a]	9[a]	18[a]	18[a]
Dichloromethane	Acetonitrile with 5% of acetic acid	42[b]	77[b]	28[b]	27[b]
Dichloromethane	Methanol	41[b]	77[b]	29[b]	27[b]
Dichloromethane	Methanol with 1% of acetic acid	42[b]	79[b]	29[b]	28[b]

[a] % Collected in washing solvent.
[b] % Collected in elution solvent.

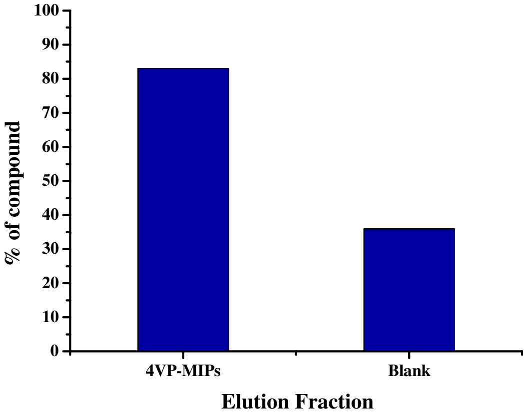

Fig. 3. Comparison of recoveries of Sudan I in elution fraction related to 4VP-MIPs column and blank column after solid phase extraction of standard solution of sudan I.

The 4-vinylpyridine shows a stronger hydrogen bond with hydroxilic group than methacrilic acid. Anyway, both polymers show a better retention of the template than the blank polymer. However, the last one has a good interaction with "Sudan I" and a significant retention of the compound was even achieved after the washing step.

An extract in chloroform of red chili powder spiked with Sudan I (0.67 μg/ml) was used to load the MISPE columns (12 ml) in order to test the ability of our best MISPE cartridges, 4Vp-MIPs, to absorb selectively "Sudan I". After the washing step with dichloromethane, the elution fraction was analyzed by HPLC. The same procedure was applied to the blank cartridges. The HPLC chromatograms of the elution fractions, for the blank column and the 4Vp-MIPs one, showed a different behaviour in the selective retention of Sudan I. In Fig. 4

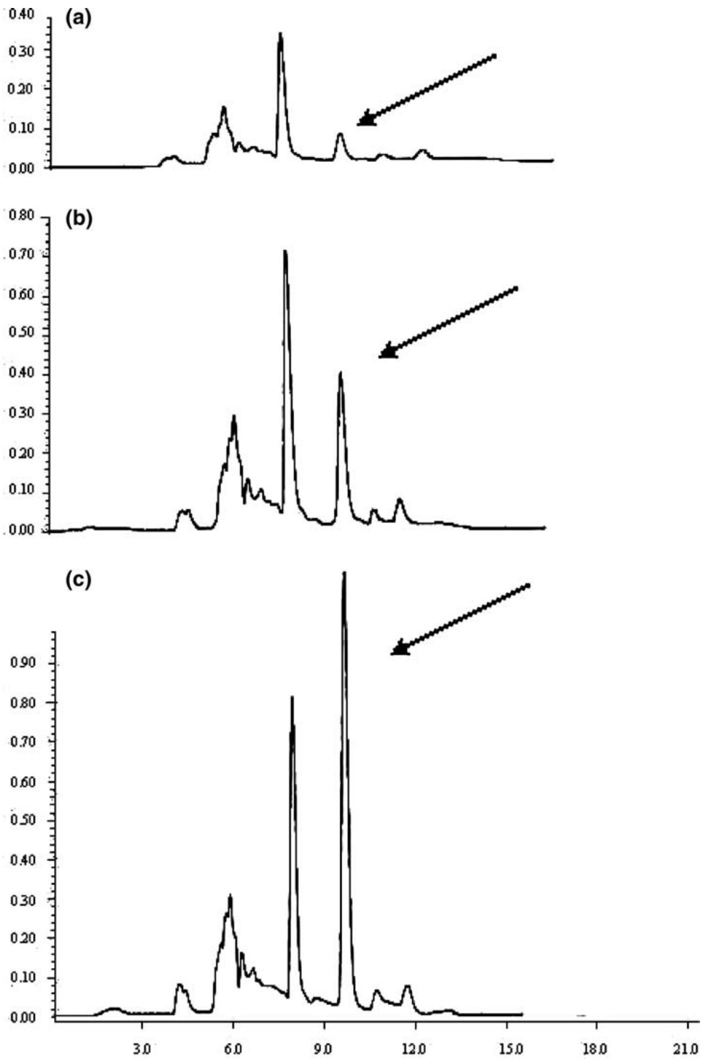

Fig. 4. HPLC choromatograms related to solution of chilli pepper spiked with "Sudan I" (a), blank cartridges (b) and to 4Vp-MIPs cartridges (c). The Sudan I peak is indicated with an arrow in the chromatograms.

it is possible to note the different intensity of Sudan I peaks of the elution fraction related to the blank column and the 4Vp-MIPs one.

The measure of total loss of Sudan I in the washing fractions amounted respectively to 10% for the MISPE column and 18% for the blank column. Instead the recovery in the elution step is 70% for the MISPE one and 30% for the blank one.

4. Conclusion

The aim of this work was the development of Sudan I (Fig. 1) MISPE and the evaluation of their performance within the frame of an analytical strategy to monitor the illegal use of this compound. The results on food matrix suggest that such purification can be considered a practicable solution for sample preparation in routine analysis when traces amount of Sudan I are no detectable using only HPLC. Further work will be the optimization of the loading, washing and elution steps.

Acknowledgement

This work was financially supported by University and Italian Murst funds.

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