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Determination of triclosan in personal health care products by liquid chromatography (HPLC)

A. Piccoli [a], J. Fiori [a], V. Andrisano [a,*], M. Orioli [b]

[a] *Dipartimento di Scienze Farmaceutiche, Università degli Studi di Bologna, Via Belmeloro 6, 40126 Bologna, Italy*
[b] *Istituto Chimico Farmaceutico Tossicologico, Università di Milano, Viale Abruzzi 42, 20131 Milan, Italy*

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Abstract

An isocratic reversed-phase liquid chromatographic (HPLC) method is proposed for the practical and reliable determination of triclosan, an antimicrobic agent incorporated into a variety of personal heath care products. Chromatographic separations were performed on a C-18 column using acetonitrile–TEA phosphate (70 mM; pH 3.5) 55:45 (v/v) as mobile phase and UV detection at 230 and 280 nm. The selectivity of the method was assured by the on-line photodiode array detector. The identity of the triclosan peak was also confirmed by HPLC MS. The method was successfully applied to the determination of triclosan in commercially available health care products (deodorant stick, dentifrice gel, mouthrinse, toothpaste and handwash). All the products displayed triclosan concentrations in compliance with the EEC directive ($\leq 0.3\%$). © 2002 Published by Éditions scientifiques et médicales Elsevier SAS.

Keywords: Liquid chromatography (HPLC); Triclosan; Cosmetics; Health care products

1. Introduction

Triclosan, (2,4,4′-trichloro-2′-hydroxydiphenyl ether) (Fig. 1) is a nonionic, broad spectrum antimicrobic agent. Because of its favourable safety profile, it has been incorporated into a variety of many personal care products, including deodorant soaps and sticks, handwash, and also mouthrinse and dentifrice for providing control of dental plaque and breath odour [1–5]. According to the European Economic Community (EEC) directive 76/768 (Annex VI and subsequent amendments) [6], its use is permitted at a maximum concentration of 0.3% (w/w). Therefore, analytical methods able to perform selective and sensitive determination of triclosan in complex formulations are necessary to verify the adherence of the finished products to the ECC legislation.

Methods generally based on reversed phase liquid chromatography (HPLC) [7–13] have been reported for the determination of triclosan and other preservatives in cosmetics, under isocratic [7–10] and gradient [10–13] conditions. The complex composition of the cosmetic formulations calls for a careful preliminary sample preparation to remove the components which could interfere with the analyte determination. The reported methods, generally developed for screening purposes, include solvent extraction procedures with a high percent content of organic solvent; hence, these solvent systems suffer from poor selectivity and can be responsible for the injection on column of significant amounts of lipophilic components, deleterious for the analytical column performance and lifetime. A more selective approach, based on supercritical fluid extraction (SFE) using carbon dioxide, has been proposed [9]; the method allows the automation of the extraction process, but involves the use of an expensive SFE apparatus, usually not available in a common quality control laboratory.

The present study was aimed at developing a practical HPLC method suitable for the routinely, selective analysis of triclosan in a variety of commercially available cosmetic preparations. To this end the sample preparation was accomplished by a simple liquid extraction with the same solvent system used as mobile phase to perform the required HPLC separations under

* Corresponding author.
E-mail address: analit@alma.unibo.it (V. Andrisano).

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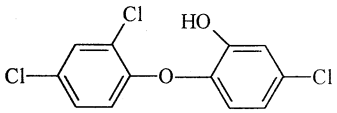

Fig. 1. Structure of triclosan.

Table 1
Assay results for the HPLC determination of triclosan in representative commercial health care products

Product [a]	Formulation	Amount taken (mg)	% Found (± SD)
A	deodorant stick	120	0.300 (0.006)
B	dentifrice (Theramed)	170	0.180 (0.004)
C	antiplaque mouthrinse	500	0.043 (0.0006)
D	toothpaste	130	0.150 (0.007)
E	handwashes	250	0.145 (0.002)

The results are the average of five analyses and are expressed as % found of triclosan in the examined products.

[a] Other ingredients: A: dipropylene glycol, glycerin, propylene glycol, stearic acid, parfume, methacrylate/stirene copolymer; B: glycerin, sorbitol, hydrated silica, alcohol, sodium laurilsulfate, aroma, PEG 32, PEG 30, glycerin stearate, sodium fluoride, disodium phosphate, magnesium sulfate, zinc sulfate, xantangum, cocamidopropyl betaine, sodium saccharin, sodium benzoate; C: propylene glycol, PEG 40, hydrogenate castor oil, sodium benzoate, sodium laurylsulfate, polysorbate 20, aroma, lactic acid, sodium lactate, sodium bicarbonate, sodium fluoride, Centella asiatica, 2-bromo-2-nitropropane 1,2-diol, sodium saccharin; D: calcium carbonate, sorbitol, glycerin, sodium lauryl sulfate, flavour, hydrated silica, sodium monofluoro phosphate, carrageenan, cellulose gum, sodium saccharin, hydroxyethylcellulose, calcium glycerophosphate, PVC; E: coco glucoside, cocamidopropyl betaine, sodium chloride, PEG 10, soyl sterol, squalane, lecithin, ascorbyl palmitate, tochopherol, glycerin, polyquaternium-7, PEG 7 glyceryl cocoate, parfume, isostereamide MIPA, sodium methacrylate/styrene copolymer, tetrasodium EDTA, lactic acid, methylchloroisothiazolinone, methylisothiazolinone.

isocratic reversed phase (C-18) conditions. The intrinsic selectivity of the HPLC method was enhanced by the use of a Diode Array detector which provided UV spectra for each chromatographic peak. The identity of the triclosan peak was also confirmed by HPLC MS and MS/MS. The proposed method was, therefore, applied to the quality control of representative commercial health care products, such as deodorant sticks, handwash, mouthrinse, dentifrice gel and toothpaste.

2. Experimental

2.1. Chemicals

Triclosan was a kind gift from Bottega Verde (Colorno, PR, Italy) and anthrone (the internal standard) was obtained from Aldrich Chimica (Italy). Acetonitrile of HPLC grade, triethylamine (TEA) and all other chemicals were from C. Erba Reagenti (Milan, Italy). Water obtained from Milli-RX apparatus (Millipore, USA) was used to prepare solutions and buffers, which were filtered through 0.45 μm Millipore filters prior to use. The sample solutions were filtered through 0.2 μm GyroDisc filters (Orange Scientific, Waterloo, Belgium) before the injection into the chromatograph.

2.2. Apparatus

The HPLC apparatus comprised a HP quaternary pump 1050 Ti series, a 7125 Rheodyne injection valve (20 μl loop), and a HP 1050 photodiode array detector (DAD) connected to a workstation HP Vectra UL 26/66. The chromatographic separations were performed on a 3 μm Waters Xterra MS C-18 (150 × 3.0 mm i.d.) column, using a mobile phase consisting of acetonitrile–TEA phosphate (70 mM; pH 3.5) 55:45 (v/v) at a flow rate of 0.4 ml/min. UV detection at 230, 254 and 280 nm was used. When HPLC MS analysis was carried out TEA phosphate was replaced by TEA acetate in the mobile phase under the same conditions. The HPLC MS apparatus consisted of a LCQ Duo (ThermoQuest, Finningam) mass detector, operating in ESI mode (70 eV), with an ion trap analyser, connected to a Jasco PU-1585 HPLC micro pump system. A Sonarex super RK 102 (35 KMz) Bandelin (Berlin, Germany) equipment with thermostatically controlled heating (30–80 °C) was used for ultrasonication.

2.3. Calibration graphs

Stock solution of triclosan (0.4 mg/ml) was prepared in the mobile phase acetonitrile–70 mM TEA buffer (pH 3.5) solution 55:45 (v/v) and stock solution of anthrone (the internal standard) in acetonitrile (0.3 mg/ml). These solutions were then used to prepare standard solutions of triclosan (0.008–0.06 mg/ml) containing a fixed concentration (0.03 mg/ml) of the internal standard (anthrone), diluting to volume with the mobile phase. Each standard solution was injected in triplicate into the chromatograph; the peak area ratios (triclosan to internal standard) were plotted against the corresponding triclosan concentrations to obtain the calibration graph.

2.4. Analysis of commercial products

An accurately weighed quantity of cosmetic sample (Table 1) was treated with 9.0 ml of the mobile phase under ultrasonication at 60 °C for 15 min. After cooling, the solution/suspension was filtered through a 0.2 μm RC15 filter using a syringe; then 1 ml of the internal standard stock solution was added to the filtrate and the solution was diluted to 10 ml with the mobile phase.

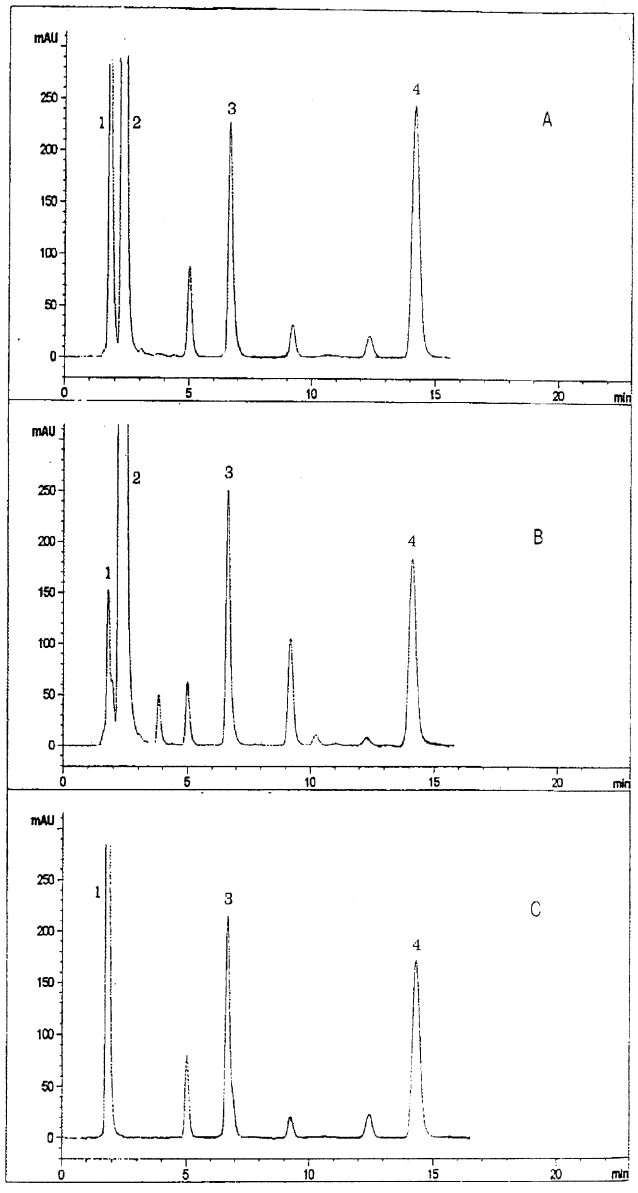

Fig. 2. Typical HPLC chromatograms obtained from: (A) dentifrice (Theramed); (B) antiplaque mouthrinse; (C) toothpaste. Peak identification: 1 = sodium saccharin, 2 = sodium benzoate, 3 = anthrone (internal standard), 4 = triclosan. For HPLC conditions see text.

The resulting sample solution was then subjected to HPLC analysis. The content of triclosan in each analysed sample was calculated by comparison with a standard solution (0.040 mg/ml).

2.5. Recovery studies

Known quantities of triclosan were added to the commercial samples and the fortified samples were subjected to the described extraction procedure and HPLC analysis. In particular, products A, C and D (Table 1) were respectively fortified at the level of 125, 180 and 130%, compared to the found content in the commercial formulation.

3. Results and discussion

The study was aimed to the following main objectives. (a) Development of a practical and selective HPLC method suitable for a reliable analysis of triclosan, and (b) application of the method to the quality control of commercial health care products.

3.1. Chromatography and detection

Reversed phase chromatographic conditions were found suitable to modulate the retention of the lipophilic analyte triclosan. Moreover, an acidic (pH 3.5) buffer was preferentially used in order to suppress the ionisation of acidic sample components in the mobile phase. Complete separation of triclosan from the other formulation components (preservatives, sweeteners) was achieved using a binary mixture of acetonitrile–70 mM TEA phosphate buffer (pH 3.5) 55:45 (v/v) in combination with a XTerra MS C-18 column, a stationary phase suitable also for mass spectrometry applications. Isocratic elution mode was applied, allowing reasonable analysis times (about 20 min) without the need of replicate column equilibration typical of the gradient elution. Representative HPLC chromatograms obtained from the analysed commercial cosmetic products are reported in Fig. 2A–C. As shown, triclosan peak (tr = 14.2 min) was separated from the other components and from the internal standard anthrone (tr = 6.6). The chromatographic peaks were identified by comparing the peak retention time and the corresponding UV spectra with those of the reference standards.

In order to improve the method selectivity and achieve additional information useful to confirm the presence of triclosan in the examined samples, liquid chromatography was combined with electrospray ionisation mass spectrometry (ESI MS) and MS/MS analysis was performed. The mass spectrum, recorded within 80–350 m/z (positive ions), showed molecular mass at 289.5 m/z (19%) and 288.4 m/z (100%) (capillary voltage 30 V, capillary temperature 180 °C). Subsequent MS/MS analysis of these ions provided mass spectra with fragments at 270.3 m/z and 252.5 (normalised collision energy 30%). Under established conditions the ESI MS detection was of great support to confirm the analyte identification in the commercial products. In a previous paper [14] conventional electron impact mass spectrometry in combination with collision spectroscopy (CAD MIKE) was applied to the identification of triclosan in various cosmetic products.

For routine analyses, however, the HPLC-DAD technique can be considered quite adequate to assure a reliable quality control of triclosan-based products; this approach was in fact used in the present application.

3.2. Analysis of commercial products

A number of commercially available cosmetics are labelled to contain triclosan. In the present work five different representative preparations were chosen to verify the applicability of the proposed HPLC method. Under the described chromatographic condition a linear calibration graph was obtained between the peak area ratio (analyte to internal standard) (Y) and the triclosan concentration C (0.008–0.06 mg/ml): $Y = 48.50 (\pm 0.79) C + 0.0077 (\pm 0.030)$ ($n = 6$; correlation coefficient 0.9995). Anthrone, eluting at a retention time of about 6.65 min, without interference from the other components of the analysed samples, proved to be a suitable internal standard. The intra-day precision (repeatability) at levels of 0.02 and 0.04 mg/ml of triclosan was satisfactory, as indicated by the relative standard deviation: (1.6 and 1.4%, respectively) of the peak area ratio, after replicate ($n = 5$) injections of the same standard solution.. The detection limit (LOD), obtained from the linear regression equation [15] was 2 μg/ml.

When the commercial products were analysed, preliminary sample preparation was accomplished by liquid extraction with the mobile phase under ultrasonication followed by filtration through a 0.2 μm filter which provided clear sample solutions. The procedure allowed most of the lipophilic excipients to be removed from the sample. The performance of the extraction step was ascertained by recovery studies, analysing fortified samples. Essentially quantitative recoveries (100.2–102.4%) with adequate precision (RSD%: 2–3.8%) were obtained for each type of formulation. Thus the same extraction procedure was applied to all the commercial samples, whose amount (Table 1) was chosen according to the triclosan content. The results of the analyses are summarised in Table 1. As can be seen, all the products contain triclosan in compliance with the EEC directive. The inter-day precision of the whole method (extraction and HPLC assay) (Table 1) can be considered good for products A–C and D, and satisfactory for the product E (toothpaste).

4. Conclusion

Liquid chromatography (HPLC), under isocratic reversed-phase conditions, using diode array detection, offers the opportunity of a practical and reliable quality control of a variety of personal care products containing triclosan. The use of diode array detector allows adequate selectivity in routine analyses; HPLC MS technique can be of great utility to confirm the triclosan peak identity. All the analysed commercial products were found to contain triclosan concentration in compliance with the EEC directive.

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