Detection of Tinuvin 770, A Light Stabilizer of Plastic Materials from Dialysis Membranes, by High-Performance Liquid Chromatographic Analysis

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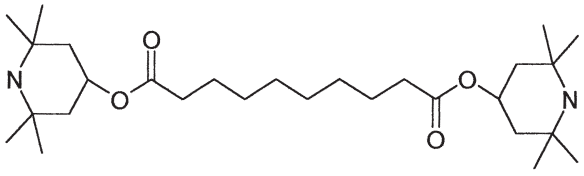
Abstract

Tinuvin 770 [bis(2,2,6,6-tetramethyl-4-piperidinyl)sebacate] is a pharmacologically active agent used worldwide as a light stabilizer for plastic materials. In vitro studies show that it is an L-type Ca^{2+} channel and neuronal nicotic acethylcholine receptor blocker. Hypotension, vegetative dysfunction, and neurological symptoms are frequently observed during a haemodialysis treatment. The release of Tinuvin 770 from plastic materials applied in haemodialysis may play a part in the development of clinical signs. In our study, four different commonly used haemodialysis membranes (polysulphon, cuprophan, and two types of hemophan) are examined. The polymers are soaked for 72 h in physiological saline solution. Isolation is carried out using a Waters Oasis SPE column for solid-phase extraction and by high-performance liquid chromatography (HPLC) with electrospray ionization–mass spectrometric detection. Tinuvin 770 release is detected from all examined membranes. Validation studies show a satisfactory selectivity, linearity, accuracy, and recovery of this method. Our results suggest that Tinuvin 770 could have specific toxicological and therapeutic importance related to haemodialysis treatment. The developed HPLC method is suitable for the detection of Tinuvin 770.

Introduction

Inhibition of degradation of plastics induced by light and oxygen has considerable technical and economical importance. Light stabilizers protect the plastics from light-induced decomposition (1). One of the most important classes of additives consists of sterically hindered amines (HALS). Their function is based on free radical scavenger properties (2). They decompose hydroperoxides, peracides, ozone, quench singlet oxygen and ketones, and form metal complexes (3,4). Tinuvin 770 (Ciba-Geigy Corp., Summit, NJ), belonging to HALS, is used worldwide as a light stabilizer of polyethylene, polypropylene, polycarbonate, polyurethane, polystyrene, polyamides, polyacetals, and acrylonitrile polymers (5,6). The chemical name of Tinuvin 770 is bis(2,2,6,6-tetramethyl-4-piperidinyl)sebacate, its structure is shown in Figure 1, and its synonyms, physical, and chemical properties are listed in Tables I–III. In consequence of the chemical mode of action, Tinuvin 770 is suitable for stabilizing thin-plastic materials (e.g., films and membranes), and its effect is independent of the thickness of the polymer layer. Tinuvin 770 is a widely applied component of plastic materials used in the medical field and also in the food industry (7). Tinuvin 770 was extracted from tubes used in the laboratory and verified as an in vitro benzothiazepin (+)-cis diltiazem-like L-type Ca^{2+}-channel blocker (8). It was also demonstrated that it inhibits neuronal nicotic-type acetylcholine receptors eluted from medical plastics. (9). Tinuvin 770 may cause a dose-dependent cytotoxicity of isolated cardiomyocytes (10). The release of Tinuvin 770 from plastics (e.g., syringes, tubes, or dialysis capillaries) into the washing fluid, infusion solutions, or directly into plasma may cause serious toxic symptoms or unexpected results in research work. Hypotension, vegetative dysfunction, and neurological signs are frequently observed during a haemodialysis treatment (11,12). Tinuvin 770 may play a role in these multifactorial clinical complications. The protection of DNA from oxidative damage by peroxyl radicals is a beneficial effect of Tinuvin 770 (13).

High-performance liquid chromatography (HPLC) is widely



Chemical Name: Bis(2,2,6,6,-tetramethyl-4-piperidyl)sebaceate
Molecular Weight = 480.74

Figure 1. Chemical name and structure of Tinuvin 770.

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used in the identification of various types of components present in plastics (14,15). The purpose of this study was twofold: the development of an HPLC method for the detection of Tinuvin 770 from medical plastic materials such as haemodialysis membranes and the determination of the viability of its solid-phase extraction (SPE) from aqueous solution. In our study, we focused on the examination of Tinuvin 770 release from four commonly used haemodialysis membranes.

Table I. Synonyms: Trade Names of Tinuvin 770 (CAS Number: 52829-07-9)

Tinuvin 770 [Decanedioic acid bis(2,2,6,6-tetramethyl-4-piperidiyl)ester]	
ADK Stab LA 77	Sanol LS 770
HALS 1	Sumisorb 577
HALS 770	T 770
LS 770	TIN 770
Mark LA 77	Tinuvin 770 DF
Sanol	Tinuvin 770 LS
Sanol 770	TK-10665
Sanol LS 700	

Table II. Physical and Chemical Properties of Tinuvin 770*

Form	White to slightly off-white granules
Odor	None
Melting point range	81–85°C
Boiling point range	Not applicable
Relative density	1.05 g/cm^3 at 20°C
Flash point	> 150°C DIN at 20°C
Ignition	330°C BAM
Oxidizing propreties	Not tested
Autoflammability	Not tested
Water solubility	< 1 mg/L at 20°C
Vapor pressure	1.3 × 10^{-8} Pa at 20°C
Partition coefficient (*n*-octanol–water)	Long Pow 0.35
pH	9.67 (1% suspension in water)
Viscosity	Not applicable
Explosive propreties	Not tested

* Material Safety Data Sheet Directive 91/155/EEC, CIBA Speciality Chemicals Inc., Additive Division, Basle, Switzerland.

Table III. Solubility of Tinuvin 770 at 20°C*

Solvent	Solubility [Tinuvin 770 (g)–solvent 100 g]
Acetone	19
Benzene	46
Chloroform	45
Acetic acid ethyl ester	24
Hexane	5
Methanol	38
Methylene chloride	56
Water	< 0.01

* Material Safety Data Sheet Directive 91/155/EEC, CIBA Speciality Chemicals Inc., Additive Division, Basle, Switzerland.

Experimental

Chemicals and reagents

Solvents and chemicals were purchased from commercial sources in the highest available purity. HPLC-grade water, methanol, and acetonitrile were obtained from Riedel de Haen (Seelze, Germany), ammonium acetate and ammonia solution from Reanal (Budapest, Hungary), hydrochloric acid from Carlo Erba (Rodano, Italy), and 0.09% saline solution (Salsol) from Human (Gödöllo, Hungary). Tinuvin 770 (molecular weight, 480.73) was obtained from Ciba-Geigy Corp. (Basel, Switzerland).

HPLC apparatus

A Shimadzu HPLC system (model 2010, Kyoto, Japan) consisting of dual pumps (LC-10ADVP), auto injector (SIL-10ADVP), column oven (CTO-10ASVP), degasser (DGU-14A), system controller (SCL-10AVP), and mass spectrometer (LCMS-2010) was used throughout this study. Data were acquired and analyzed on a computer (Compaq Deskpro, Agilent, Palo Alto, CA) using LabSolution LCMS solution program (Shimadzu).

Sample preparation for HPLC analysis

Four different types of haemodialysis membranes were analyzed: polysulphon (UF 5,5, Fresenius Medical Care, Bad

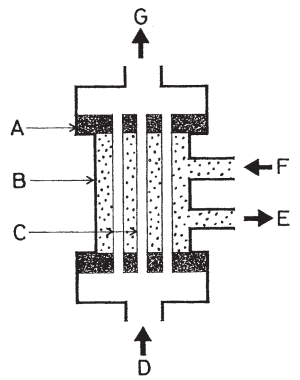

Figure 2. Cross-section of a haemodialysator: (A) potting compound, (B) jacket material, (C) dialysis membrane, (D and G) blood circulation, and (F and E) flow of dialysis fluid.

Table IV. Tinuvin 770 Concentration in Physiological Saline Solution after 72-h Dipping, Measured by HPLC–MS (*n* = 3 each)

Type of Membrane	ng/g
Polysulphon	484 ± 7.3
Curophan	73.3 ± 1.2
Hemophan, HG	37.3 ± 0.7
Hemophan, Lundia	31.0 ± 0.6

Homburg, Germany), cuprophan (Lundia Alfa, Gambro, Lund, Sweden), and two types of hemophans (Lundia Aria, Gambro, Lund, Sweden and Hospal HG 600, Gambro-Hospal, Lund, Sweden). During the haemodialysis treatment, only the membrane material contacts with blood circulation. Therefore, the jacket materials and potting compounds of the haemodialysator were removed. A cross section of the haemodialysator is given in Figure 2. A 4-g aliquot of each membrane material was soaked in 100 mL of physiological saline solution. After 72 h, dipping isolation was carried out using SPE.

Tinuvin 770 was isolated from a 50-mL aliquot of physiological saline solution using Waters Oasis MCX 3-mL (60 mg) SPE columns (Millford, MA). The columns were conditioned with 2 mL methanol and 2 mL water. The samples were loaded onto the column, fixed with 2 mL 0.1M HCl, washed with 2 mL methanol, and eluted with 3 mL methanol and 1 mL NH_4OH (25 %). The eluate was evaporated to dryness under nitrogen at room temperature and redissolved in 1 mL of methanol. A 20-µL aliquot of this solution was injected into the HPLC.

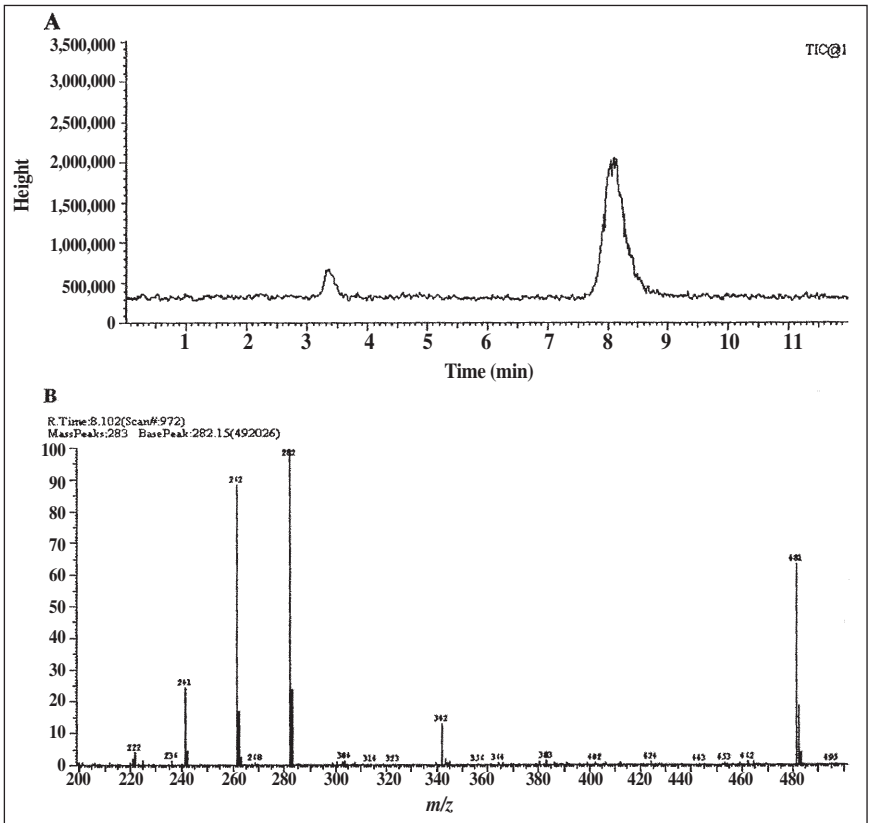

Figure 3. HPLC–MS chromatogram of Tinuvin 770: (A) MS chromatogram and (B) MS spectrum (scan mode).

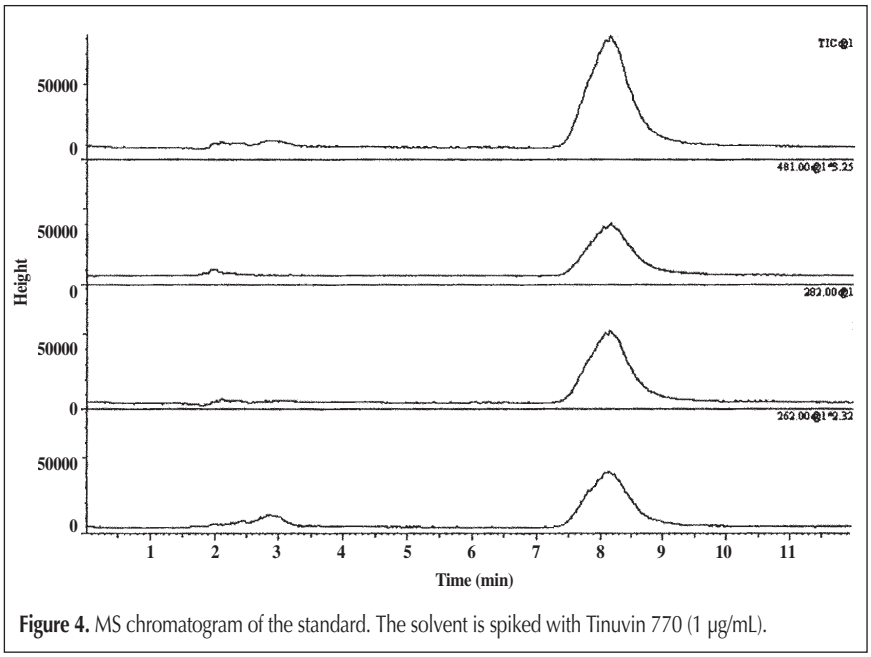

Figure 4. MS chromatogram of the standard. The solvent is spiked with Tinuvin 770 (1 µg/mL).

Chromatographic condition

Chromatographic isolation was achieved on a 125- × 2-mm-i.d. column containing Superspher 60 RP-select B (Merck, Darmstadt, Germany). The mobile phase consisted of a 20:80 (n/n) mixture of ammonium-acetate buffer (pH 4.05) and acetonitrile at a flow rate of 0.3 mL/min.

The liquid chromatography (LC)–mass spectrometry (MS) system was used in the positive ionization mode, and we observed the protonated molecular ion (*m/z* 481) (Figure 3). The routine time needed for assay and system suitability test was 12 min.

Results and Discussion

Specificity

The previously described SPE isolation resulted in excellent separation of this plastic additive. Under the given conditions, the HPLC–MS peak of Tinuvin 770 was detected in both the standards and samples (Figure 4–6). This peak was symmetrical, well resolved, and its retention time was reproducible. The HPLC chromatogram demonstrates the satisfactory result achieved in the analysis of Tinuvin 770, and the obtained data allow qualitative and quantitative analyses (Table IV).

Linearity

Linearity was validated by measuring the area responses at three injected quantities: 4, 40, and 400 ng (Figure 7). The response, expressed as the correlation coefficient (r^2) showed good linearity ($r^2 \geq 0.9985$) in a wide concentration range.

Precision

The repeatability of this method was evaluated on the basis of five replicate analyses of the standard (40 ng of Tinuvin 770). The peak's

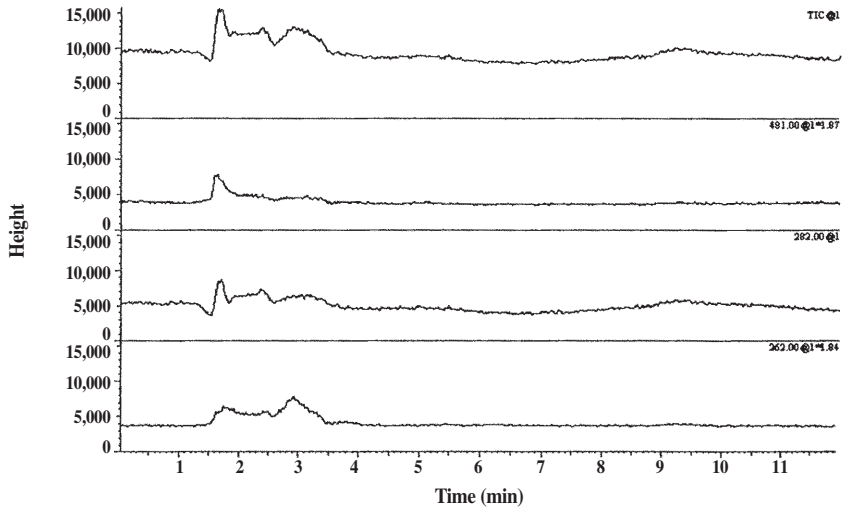

Figure 5. MS chromatogram of HPLC-grade water (negative control).

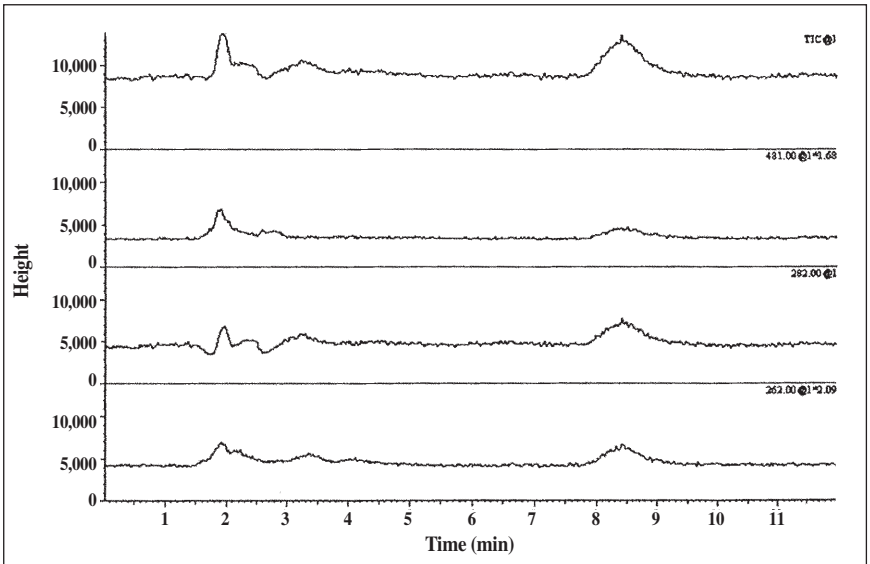

Figure 6. MS chromatogram of a cuprophan sample after SPE extraction. The MS chromatogram indicates the presence of a Tinuvin 770 peak from the washing solution (single ion monitoring mode).

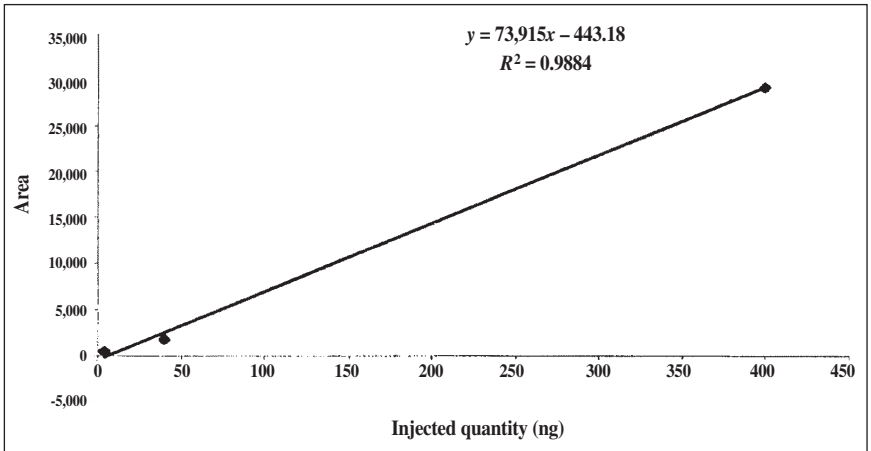

Figure 7. Linear regression plot for the linearity validation of the assay of Tinuvin 770.

retention time (t_R) showed a relative standard deviation of ≤ 0.5%, and similar precision values were obtained for actual samples.

Accuracy

Accuracy was validated by spiking the media with Tinuvin 770 at different levels. The recovery values (mean ± standard deviation) were 91.6% ± 1.8%. The slopes of these regression lines did not differ from the calibration curve.

Limits of detection and quantitation

The detection limit (DL) and quantitation limit (QL) for Tinuvin 770 were determined. The DL was 1 ng/mL and the QL was 10 ng/mL. The results indicate that the method is very sensitive.

Conclusion

An SPE and HPLC–MS method was developed and validated for the determination of Tinuvin 770 from aqueous solution. This method is specific, sensitive, accurate, and linear in assaying the examined plastic additive. The simplicity and reliability of this method permits its application in routine toxicological control and pharmaceutical assay and allows the rapid qualitative and quantitative detection of Tinuvin 770, including in trace analysis. Regular toxicological control may also be necessary for the safe use of plastics containing this additive used in medical, laboratory, and food materials. The results obtained from the analysis of the washing solution of haemodialysis membranes indicate the necessity of the analysis of the serum of haemodialysed patients. According to our results, the physiological saline solution can be regarded as a plasma-like solvent that has the ability to dissolve Tinuvin 770 from haemodialysis membranes. In our further study, we plan to develop a method for the detection of Tinuvin 770 from a biological matrix (blood, serum, and urine).

Acknowledgments

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