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Determination of rotenone in river water utilizing packed capillary column switching liquid chromatography with UV and time-of-flight mass spectrometric detection

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Abstract

Fast and sensitive packed capillary column switching liquid chromatography methodology has been developed for the determination of the pesticide rotenone in river water. Sample volumes of up to 1 ml are loaded onto a 23×0.25 mm, 5 μm Kromasil C_{18} packed capillary precolumn using a noneluting solvent composition of water–acetonitrile (99:1, v/v) at flow-rates up to 100 μl/min prior to solute backflushing onto a 200×0.32 mm, 3.5 μm Kromasil C_{18} packed capillary analytical column using a mobile phase of water–acetonitrile (30:70, v/v) at a flow-rate of 5 μl/min. The method was evaluated using river water samples spiked with rotenone in the concentration range 0.5–50 ng/ml using UV detection. The within-assay precision was between 5.0 and 7.7% relative standard deviation (RSD, $n=6$) and the between assay precision was between 7.5 and 8.9% RSD ($n=6$). The method was linear within the investigated mass range displaying a calibration curve correlation factor of 0.997. The mass limit of detection was 10 pg corresponding to a concentration limit of detection of 10 pg/ml, using time-of-flight mass spectrometry.

Keywords: Water analysis; Column switching; Large volume injection; On-column focusing; Packed capillary columns; Pesticides; Rotenone

1. Introduction

Rotenone is a pesticide of botanical origin that is used in rivers or river systems to exterminate the parasite *Gyrodactylus salaris (G. salaris)* which lives and propagates mainly on salmon fry. The North Atlantic salmon is particularly sensitive towards *G. Salaris* and, with few exceptions, all salmon fry will die after the introduction of *G. Salaris* to the river.

Rotenone is toxic to all living organisms, and particularly organisms with gills will absorb this pesticide quickly. Rotenone is a hydrophobic compound (Fig. 4) and dissolves in the cell membrane as well as the inner membrane of the mitochondria [1]. Rotenone acts by inhibiting NADH:ubiquinone oxidoreductase (in complex 1 of the electron transport chain), terminating the transport of electrons to O_2 and consequently the synthesis of adenosine triphosphate (ATP) which is fatal for the organism [1].

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Hence, by introducing rotenone to the infected river, all fish will be die resulting in the extermination of the parasite due to the lack of a host. Twenty-four out of forty infected rivers in Norway have been treated with rotenone during the last 5–10 years and eleven of the rivers are presently declared free of the parasite and the salmon population has been restored [2].

Due to its toxicity towards fish, there is a need for monitoring rotenone in water [3–5]. The determination is usually performed using conventional liquid chromatography (LC) with UV or mass spectrometric detection and off-line preconcentration steps [3–5]. A method for measuring rotenone in raw honey has also been published [6].

Packed capillary LC (PCLC) has in recent studies proven itself to be a very suitable technique for the determination of low concentrations of compounds compared to conventional LC [7–10] due to the potential increase in mass sensitivity. Furthermore, even though most mass spectrometers employing ESI nowadays can handle mobile phase flow-rates up to 1–2 ml/min, flow-splitting is often required when using conventional LC, due to the fact that most instruments operate more favorable at flow-rates not exceeding 0.2 ml/min [11]. In addition, applying flow-rates of 1–2 ml/min requires more maintenance of the ESI source, especially when nonvolatile additives are included in the mobile phase [11].

Numerous organic compounds are often present in very low concentrations in aqueous matrices making detection and quantification of such compounds a challenging task. A possible solution to this problem is to inject large sample volumes or even the total sample volume, making available a concentration limit of detection (cLOD) improvement factor of several orders of magnitude compared to when using a typical PCLC injection volume. However, injection of large sample volumes in miniaturized LC is a time consuming process, due to the low flow-rates usually employed with this technique, typically in the range of 1–10 μl/min [8,11–13]. In addition, solute focusing is a prerequisite for large volume injections. Thus, time-consuming preconcentration steps are often inevitable to facilitate detection of compounds of biological and environmental interest. The concept of column switching and large volume injections in PCLC was introduced by Chervet et al. [14], and has been explored successfully by several authors [7,15–26]. The present study displays a capillary column switching system where sample volumes up to 1 ml are loaded onto a short enrichment column, allowing sample loading at flow-rates up to 100 μl/min, prior to column-switching and backflushed solute elution onto the analytical column at a linear velocity close to optimal.

The coupling of time-of-flight mass spectrometry (TOF-MS) with electrospray ionization (ESI) has gained popularity due to its sensitivity, theoretically unlimited mass range, high mass resolution and the capability of highly accurate mass determinations [27–30]. The TOF-MS is not a scanning instrument but detects ions over the whole m/z range practically at the same time. This feature makes the TOF-MS very suitable as the sensitivity is enhanced compared to scanning instruments when monitoring more than one m/z value. In addition, the possibility of extracting selected ion chromatograms (SIC) from the total ion chromatogram (TIC) makes the TOF-MS a very selective and sensitive detector. However, MS instrumentation is still quite expensive and not yet present in all laboratories working with water quality monitoring. Hence, a UV detector was connected online with the TOF-MS in the present study, and was used for method evaluation due to its stability and ease of operation.

Rotenone-containing water is not approved for human consumption. Thus, the aim of this study was to develop PCLC methodology for fast and sensitive determination of the hydrophobic compound rotenone in river water utilizing packed capillary precolumns and large volume injection, eliminating the need for extensive offline sample enrichment.

2. Experimental

2.1. Materials and reagents

HPLC grade acetonitrile (ACN) and formamide, methanol (MeOH) and chloroform were obtained from Rathburn (Walkerburn, UK), Mallinckroft Baker (Deventer, The Netherlands) and Merck (Darmstadt, Germany), respectively. Technical potassium silicate was obtained from Merck. Water used to prepare the mobile phases was obtained from

a Milli-Q ultrapure water purification system (Millipore, Bedford, MA, USA). Nitrogen (99.99%) was purchased from AGA (Norway). All fused-silica capillaries were obtained from Polymicro Technologies (Phoenix, AZ, USA). Rotenone was obtained from Sigma–Aldrich (Steinheim, Germany).

2.2. Sample preparation

Stock solutions of 0.1 g rotenone/l MeOH were prepared from which spike solutions were made by appropriate dilution with water obtained from an untreated river containing a salmon population. Due to the known photochemical oxidation and acidic hydrolysis of rotenone in aqueous solutions [3], all solutions containing rotenone were kept dark in sealed vials at 4 °C between injections. The methanol stock solutions were stored under argon. The river water used to make the spike solutions was filtered through a Millex-GV 0.22-μm filter unit (Millipore) prior to injection. The following rotenone validation solutions were prepared: 0.5 μg/l ($n=6$), 5 μg/l ($n=1$), 10 μg/l ($n=6$), 25 μg/l ($n=1$), 50 μg/l ($n=6$).

2.3. Column preparation

The capillary columns were slurry packed using ACN–water (70:30, v/v). The analytical columns of 0.32 mm I.D. and 0.45 mm O.D. were packed in lengths of 20 cm, and the stationary phase material was 3.5 μm Kromasil C_{18} (Eka Nobel, Bohus, Sweden). Valco ZU1C unions in combination with Valco FS1.4 polyimide ferrules and Valco 2SR1 steel screens served as column end fittings. The precolumns of 0.25 mm I.D. and 0.375 mm O.D. were packed with Kromasil C_{18} 5-μm particles in lengths of 10 cm and cut to a length of 23 mm. Prior to precolumn packing a ceramic frit was prepared at the outlet of the column to serve as packing material restriction using a mixture of potassium silicate and formamide, according to a procedure described elsewhere [31]. The frit was cut to a length of 0.3 mm prior to packing. A Valco ZU.5 union with an FSR.4–5 adapter and a steel screen served as column end fitting at the opposite end.

2.4. Chromatographic system

A Hitachi L-7110 (Merck) isocratic LC pump served as the mobile phase delivery system (pump 1), while a Waters 590 LC pump provided the sample loading (pump 2). A Valco model C4 injection valve or a model 7725 injection valve (Rheodyne, Cotati, CA, USA) (valve 1) was used for manual injections of sample volumes of 0.05–1000 μl. A M-435-1 six-port low-dispersion micro injection valve from Upchurch Scientific (Oak Harbour, WA, USA) served as column-switching valve (valve 2). The mobile phase consisted of ACN–water (70:30, v/v) at a flow of 5 μl/min, while the sample loading solution was Milli-Q water with 1% ACN pumped at flow-rates of 5–100 μl/min.

The precolumn end containing the ceramic frit was mounted directly in port 1 of valve 2, while the other end was mounted in port 4 through a 7-cm, 100-μm I.D. fused-silica capillary. A 50-μm I.D. fused-silica capillary of 15 cm length was used to connect valves 1 and 2. The analytical column was connected to valve 2 through a 10 cm, 30 μm I.D. fused-silica capillary and the column outlet was connected to a Spectra 100 UV detector from Spectra Physics (Spectra-Physics, Fremont, CA, USA) through a 10 cm, 30 μm I.D. fused-silica capillary. The UV detector was equipped with an UZ-LI-CAP z-cell from LC Packings (Amsterdam, The Netherlands) with 8 mm optical pathlength and was operated at 294 nm. A schematic description of the column switching system is presented in Fig. 1.

The outlet of the UV detector was directly connected to the ESI source of the TOF-MS, which was a Micromass LCT instrument equipped with a Z-spray atmospheric pressure ionization source for ESI (Micromass, Manchester, UK). The operating conditions for the MS were as follows: extraction cone:

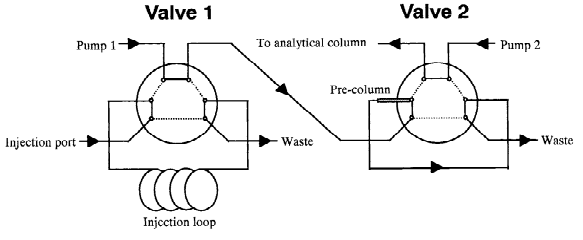

Fig. 1. Schematic diagram of the column switching system.

6 V, sample cone: 50 V, capillary: 2500 V, source temperature: 100 °C, desolvation temperature: 120 °C. Mass spectra were recorded in the positive ion mode over the m/z range 100–700. The MS was controlled and data were acquired using MASSLYNX v3.5 software.

3. Results and discussion

Trace determination of hydrophobic compounds in water intended for human consumption is a very important but often time consuming task, usually requiring a number of preconcentration steps prior to the final quantification step. By the use of column switching PCLC, low concentrations of hydrophobic compounds of interest can potentially be determined without the need for extensive offline preconcentration steps, when applying focusing techniques upon injection.

3.1. Mobile and stationary phase considerations

Whether or not analyte focusing is successful totally depends upon the analyte being completely retained on the precolumn. Inadequate focusing will cause band broadening resulting in diminished efficiency and resolution. Coeluting compounds are usually not crucial when employing TOF-MS detection due to the option of displaying SIC (selected ion chromatogram) which offers selective detection by tracing selected m/z values. However, introducing numerous interferences into the ion source increases the possibility of ion suppression effects resulting in reduced sensitivity [32]. Furthermore, introduction of interferences usually increases the baseline noise and hence lowers the signal-to-noise ratio.

Kromasil C_{18} 3.5-μm particles were employed as stationary phase material for the analytical column due the known high bonding density of this material [33]. The material showed adequate performance with regard to efficiency and appropriate resolution, thus no additional stationary phase materials were evaluated. Based on these facts, the same material was initially employed for the precolumn. However, the desired elevated sample loading flow-rates were not obtainable using these particles due to the high back-pressures generated by the precolumn and subsequent leaking of the switching valve. Hence, Kromasil C_{18} 5-μm particles were explored, resulting in lowered back pressure compared to the 3.5-μm particles, thereby eliminating the valve leaking problems associated with the use of 3.5-μm particles. The pressure was 300 bars when using 5-μm particles, a mobile phase flow-rate of 100 μl/min and a precolumn length of 2.3 cm. A precolumn length of 2.3 cm was necessary in order to mount the precolumn properly in the switching valve, while an analytical column length of 20 cm provided separation of rotenone from the sample components present in the water sample (Fig. 2).

The intended use of ESI-TOF-MS for detection automatically puts some limitations on the mobile phase components applicable. Studies provided by Ho et al. [3] shows the suitability of ACN as the organic component of the mobile phase in the reversed-phase LC determination of rotenone in water, and thus it was chosen for preliminary testing of the mobile phase composition. A mobile phase consisting of ACN–water (70:30, v/v) provided the best compromise between separation and time of analysis. The selected mobile phase composition is also compatible with ESI-MS.

3.2. Sample introduction and chromatography

In column switching systems, breakthrough and hence poor recoveries is a probable outcome of inadequate solute focusing. Hence, in order to evaluate the focusing of rotenone in the presented column switching system, several tests were performed.

3.2.1. Injection flow-rates

The injection of large sample volumes in PCLC is a time consuming process employing typical flow-rates of 1–10 μl/min. As an illustration, loading of a 1-ml sample solution takes 3 h and 20 min operating at 5 μl/min, which is obviously not an ideal situation. However, when loading at higher flow-rates, the possibility of solute breakthrough increases. Furthermore, high flow-rates imply high pressures, which may induce leaks in the switching valve. Cappiello et al. have recently developed a packed capillary off-line column-switching system where sample loading on the precolumn was performed at a flow-rate of 30 μl/min [26], while

Molander et al. have developed online column-switching methodology that enables sample loading flow-rates up to 40 μl/min [7,34].

Initially, water–acetonitrile (99:1, v/v) was employed to wet the ligands of the stationary phase as sample loading solution. To determine the suitability of this solvent composition, rotenone was injected using water–acetonitrile (99:1, v/v) as mobile phase. No elution of rotenone was observed within 5 h of operation and consequently, this solvent composition was employed for further injection flow-rate studies.

In order to determine the maximum sample loading flow-rates applicable in the present study, a fixed volume of 1 ml water containing 100 pg of rotenone was injected using flow-rates from 10 to 100 μl/min. Over this range (four data points; $n=3$) the peak areas remained essentially constant, viz. to within 2–3%. However, when operating at a flow-rate of 100 μl/min, valve leakage was occasionally observed, thus making this flow-rate unsuitable for routine measurements. Hence, further system testing and method evaluation were performed at 70 μl/min, which still is a considerable improvement compared to previously published results on sample loading in column-switching PCLC [7,26,34].

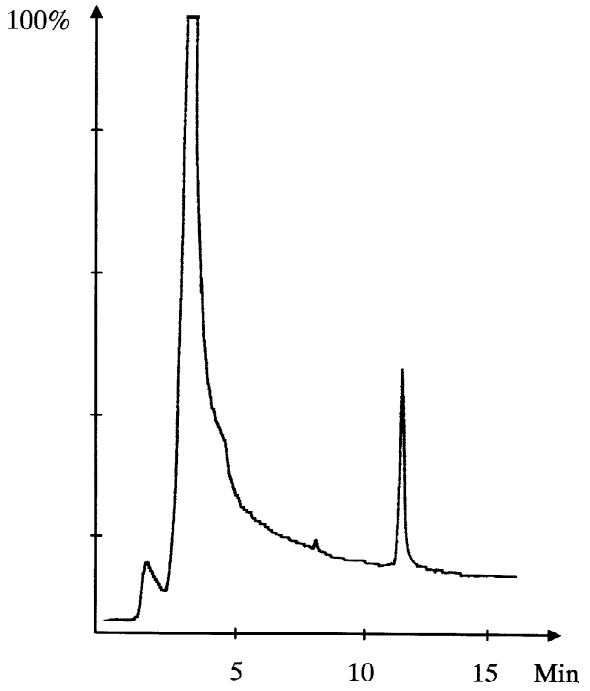

Fig. 2. Analysis of a river water sample spiked with 5 μg/l rotenone; 1 ml water was injected at a flow-rate of 70 μl/min; UV detection was at 294 nm.

3.2.2. Large volume injections

The effect of using different injection volumes upon the peak area was investigated by injecting a fixed amount of 250 pg of rotenone using sample volumes varying from 0.2 to 1000 μl at a loading flow of 70 μl/min. The peak areas (five data points; $n=3$) were found to be constant to within 4–5% over the wide range tested. This indicates that the focusing process is independent of injected sample volumes up to 1 ml.

3.3. UV and time-of-flight mass spectrometric detection

The eluted compounds were detected by a UV detector equipped with a capillary z-cell and an ESI-TOF-MS from Micromass, coupled in series as described in the experimental section. Rotenone has an absorption maximum at 210 nm and one at 294 nm, with the maximum at 210 nm having the highest molar absorptivity. Nevertheless, due to a more stable baseline obtained and more selective detection, 294 nm was employed as detection wavelength. In Fig. 2 a UV-chromatogram of 5 ng rotenone injected into the column switching system is presented. Rotenone elutes after 12 min, well separated from matrix components present in the water sample. Verification of the peak identity was performed by ESI-TOF-MS. Optimization of the MS detector response for rotenone was performed by direct infusion of a solution containing 1 μg rotenone per ml river water at a flow-rate of 5 μl/min. By varying the cone voltages and the capillary voltage the highest signal-to-noise (S/N) ratio was obtained at the conditions described in the experimental section. No in-source fragmentation was observed even at high sample cone voltage. Ionization in the negative mode was also evaluated, but showed inadequate performance compared to ionization in the positive mode with regard to S/N ratio and mass resolution. The positive ion mass spectrum (Fig. 3) of the rotenone peak consisted mainly of the molecular ion, the sodium adduct and the potassium adduct at the

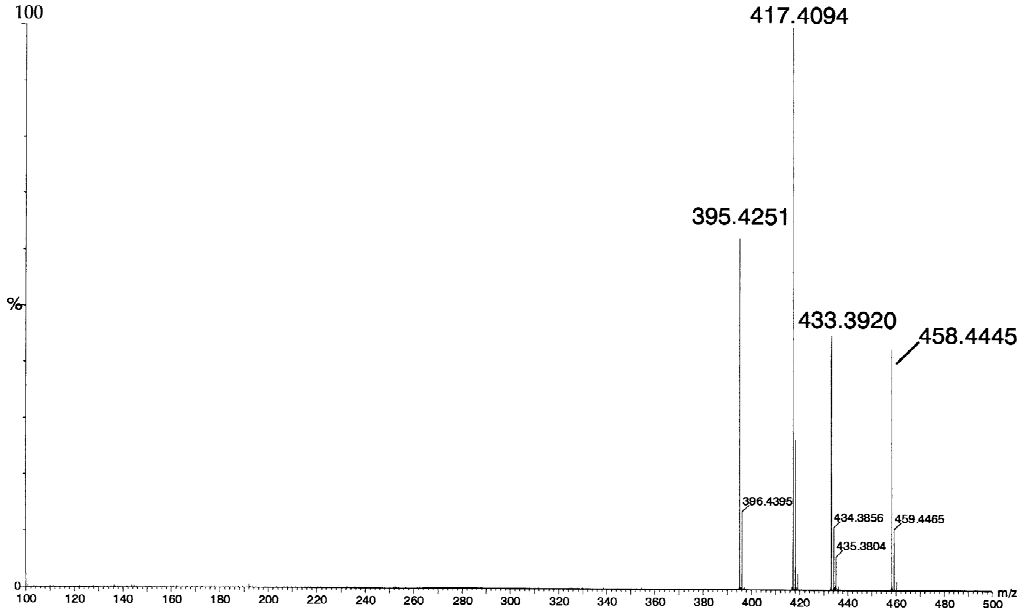

Fig. 3. Mass spectrum of the rotenone peak obtained by positive ion electrospray ionization time-of-flight mass spectrometry.

m/z values 395.4 (M+1), 417.4 (M+23) and 433.4 (M+39), respectively. Proposed identities of the ions at m/z values 435.4 and 458.4 are clusters made up of rotenone and sodium including a water or an ACN molecule, respectively. The formation of small amounts of sodium and potassium adducts in the ion source is common in ESI, but is probably intensified in the present studies due to the use of river water samples. A SIC of the m/z values 395, 417 and 433 is displayed in Fig. 4, and the matrix front observed in the UV chromatogram is almost absent.

3.4. Method evaluation

The method was evaluated by injecting water samples from the salmon river spiked with different amounts of rotenone. All injections were performed utilizing a sample loading flow-rate of 70 μl/min and an injection volume of 1 ml. Due to the high stability of the UV detector and the longer transfer line required for the ESI-TOF-MS, the results obtained utilizing the UV detector were used for method evaluation.

The mass limit of detection (mLOD) of the method was 100 pg ($S/N=3$) employing UV detection and 10 pg ($S/N=3$) employing ESI-TOF-MS, corresponding to a cLOD of 100 ng/l and 10 ng/l, respectively. This is an improvement of a factor of 40 as compared to other published results for the determination of rotenone in water [3]. The limit value of pesticides in drinking water, given by the Norwegian government, is 100 ng/l [35]. The within-assay ($n=6$) and between-assay precision (six injections on 6 different days) were determined using peak area measurements. The within-assay precision was 5.0, 7.7 and 5.6% relative standard deviation (RSD) ($n=6$) and the between assay precision was 7.5, 7.8 and 8.9% RSD ($n=6$) for levels of 0.5, 1 and 5 μg/l, respectively. The method was linear within the investigated mass range 0.5–50 ng ($n=5$), displaying a calibration curve correlation factor of 0.997. The recovery of the method was determined by injecting 200 pg of rotenone directly on the analytical column from a 50-nl loop and injecting the same amount into the column-switching system from a 0.5-ml loop. The peak areas were compared and the recovery was estimated to be 95%.

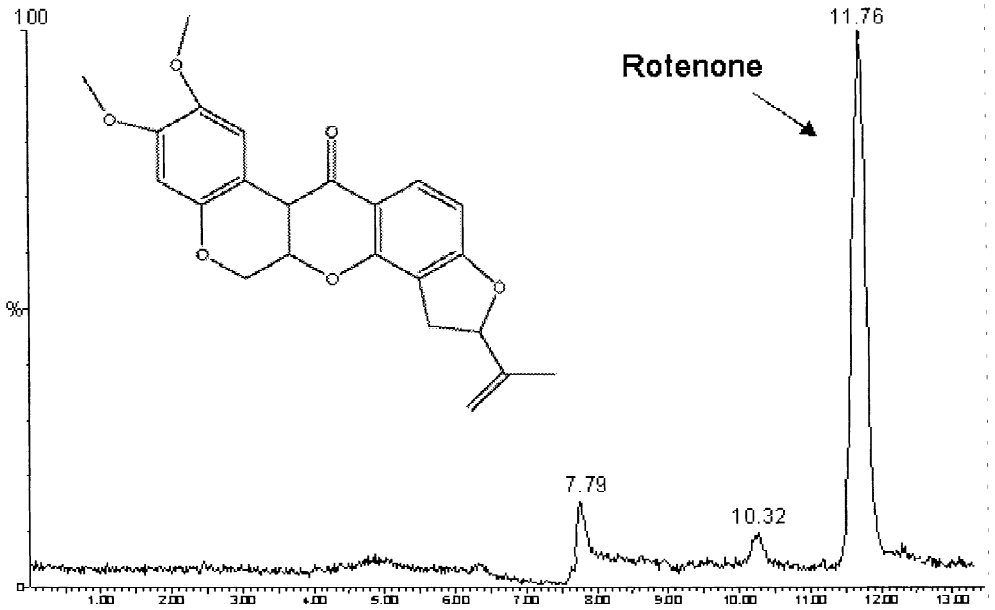

Fig. 4. Selective ion chromatogram of m/z values 395, 417, 433 of 100 ng/l of rotenone determined in river water using the developed column switching system. The structural formula of rotenone (M_w 394.4) is inserted into the chromatogram.

4. Conclusion

This work has demonstrated that packed capillary column switching liquid chromatography is a very suitable technique for the determination of rotenone in fresh water samples. The developed system offers fast loading of sample volumes up to at least 1 ml prior to backflushing onto the analytical column where determination of the component occurs. Furthermore, the developed methodology has the potential of providing an improved cLOD and more time-efficient analyses as compared to more conventional offline preconcentration methods. This column switching system should be applicable for sensitive trace determinations of a wide range of compounds present in water.

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