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Determination of hydroxy-substituted polycyclic aromatic hydrocarbons by high-performance liquid chromatography with electrochemical detection

M.T. Galceran*, E. Moyano

Departament de Quimica Analítica, Universitat de Barcelona, Diagonal 647, 08028 Barcelona, Spain

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Abstract

Conditions were established for the determination of hydroxy-substituted polynuclear aromatic hydrocarbons (hydroxy-PAHs) using high-performance liquid chromatography with electrochemical detection. Reversed-phase chromatography with methanol–aqueous phosphate buffer (pH 3.0) (50:50) was adopted for the separation of the hydroxy-PAHs. The figures of merit were calculated; the detection limits (signal-to-noise ratio = 2:1) ranged from 20 to 200 pg. The method was applied to the determination of these compounds in an aerosol sample and 5-hydroxyindane, 2-hydroxy-9-fluorenone and 2-nitro-1-naphthol were tentatively identified at the pg/m^3 level. The presence of 2-nitro-1-naphthol (0.2 ng/m^3) was confirmed by gas chromatography–mass spectrometry.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) constitute a class of organic compounds which are generated in a variety of combustion processes and released to atmosphere preferentially associated with submicron-size particles [1,2]. It has been known for more than three decades that organic extracts of the fine particulate organic matter (POM) collected in ambient urban air are carcinogenic [3,4].

When released into a polluted atmosphere, particle-adsorbed PAHs are exposed to a variety of gaseous co-pollutants. These include stable molecules and highly reactive intermediates, both free radicals and electronically excited molecular species resulting from absorption of radiation. Polar functional groups are introduced by chemical reactions with these species, giving several PAH derivatives including compounds with hydroxy, ketone, quinone and nitro substituents on the parent PAH. The transformation reactions may show seasonal variations [5]; a major pathway for PAH degradation in winter is probably the reaction with nitrogen oxides and with the resulting acids. Photochemical reactions with oxygen and reactions with secondary air pollutants such as ozone, peroxyacetyl nitrate and hydroxyl and hydroperoxyl radicals are expected to be high in summer.

It has been shown by the Ames test that the moderately polar derivatives have significant mutagenity compared with PAHs [6–8]. Nevertheless, the polar fractions of the aerosol extracts have been characterized to a limited extent. Moderately polar compounds such as nitro- and

* Corresponding author.

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oxy-PAHs have been found in aerosol samples [9–12] and the mutagenicity of the extracts has been attributed to the presence of these compounds. In addition, compounds of higher polarity, such as hydroxy-PAHs [13], nitrated lactone derivatives [14] and aliphatic and aromatic carboxylic acids and their hydroxy derivatives [15,16], have also been identified.

Although gas chromatography with selective detectors has been used in the determination of the hydroxy-PAHs [16], HPLC with electrochemical detection is an option to be considered for these relatively highly polar compounds, since high selectivity and sensitivity are achieved. To our knowledge, this technique has not been used for the determination of hydroxy-PAHs.

Electrochemical detection is only possible for compounds that have oxidizable or reducible functional groups within the potential window of the measuring electrode. For instance, reduction of oxy- and nitro-PAHs has been used for the determination of these compounds [9–12]. Furthermore, hydroxy-PAHs can be detected by oxidation with conventional solid electrodes at positive potentials. The reactions on the electrode surface (Fig. 1) are very similar to those of phenols [17].

This paper describes the application of HPLC with electrochemical detection (ED) for the determination of some hydroxy-PAHs in urban aerosols. The optimum separation conditions and working potential were established and the quality parameters were calculated. Atmospheric aerosol samples were analysed and some of the hydroxy-PAH derivatives were found at pg/m^3 levels.

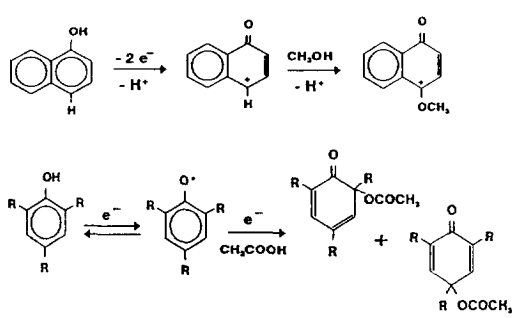


Fig. 1. Hydroxy-PAH electrochemical reactions.

2. Experimental

2.1. Chemicals

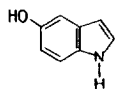
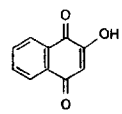
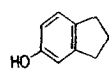
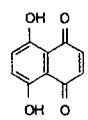
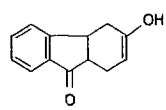
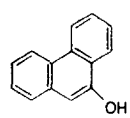
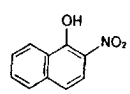
Analytical-reagent grade dichloromethane (Panreac, Barcelona, Spain), acetone (Carlo Erba, Milan, Italy) and methanol (Merck, Darmstadt, Germany) were used for the extraction of organic compounds from atmospheric aerosols. For the mobile phase, HPLC-grade methanol (Merck) and water purified with a Culligan (Barcelona, Spain) system were used. The buffer solutions were prepared with analytical-reagent grade phosphoric acid (Merck) and potassium dihydrogenphosphate (Merck). The compounds listed in Table 1 were provided by Carlo Erba, EGA Chemie (Steinhein, Germany), Fluka (Buchs, Switzerland), Janssen-Chimica (Geel, Belgium) and Merck. Bond-Elut C_{18} (500 mg) cartridges and coupling pieces were obtained from Analytichem International (ICT, Basle, Switzerland).

A stock standard solution was prepared containing 0.2 mg/ml of each in methanol, and 2,4-dibromophenol from EGA Chemie (Steinhein, Germany) was used as an internal standard.

2.2. Chromatographic conditions

HPLC was carried out with a Knauer (Bad Homburg, Germany) Model 64 pump and an ESA (Bedford, MA, USA) Coulochem 5100A detector with a dual-electrode analytical cell (ESA Model 5011) equipped with two working electrodes, a large-surface-area coulometric electrode and a high-efficiency amperometric electrode, a counter electrode and a Pd reference electrode. A 0.2-μm ESA graphite filter was placed before the analytical cell. A Merck–Hitachi D-2500 Chromato-Integrator integrator (Merck), was used. The sample was introduced by a Rheodyne (Cotati, CA, USA) Model 7125 injector with a loop of 20 μl. An RP-Select-B

Table 1
Hydroxy-PAHs studied

No.	Compound	Abbreviation	Structure
1	5-Hydroxyindole	5-HI	
2	2-Hydroxy-1,4-naphthoquinone	2-H-1,4-NQ	
3	5-Hydroxyindane	5-IOH	
4	5,8-Dihydroxy-1,4-naphthoquinone	5,8-DH-1,4-NQ	
5	2-Hydroxy-9-fluorenone	2-H-9-FLO	
6	9-Hydroxyphenanthrene	9-HF	
7	2-Nitro-1-naphthol	2-N-1-N	

(5 μm, 150 × 4 mm I.D.) reversed phase column with a Nucelosil C_{18} precolumn (5 μm, 30 × 4 mm I.D.) was used.

GC–MS was carried out with a Varian SATURN III GC/MS ion-trap detector) equipped with both a waveform generator and a Varian Model 8200 autosampler. SATURN Revision C software was used for data acquisition in the full-scan electron impact (EI) ionization mode. For the gas chromatographic separation, a DB-17 fused-silica capillary column (30 m × 0.25 mm I.D.) (J&W Scientific, Folsom, CA, USA) with a 0.25-μm film thickness was used with helium as carrier gas at a linear velocity of 30 cm/s. The temperature was held at 60°C for 1 min, programmed to 180°C at 30°C/min and then to 270°C at 10°C/min, and maintained at 270°C for 10 min. The injector, the interface and the ion-trap temperatures were 260, 250 and 270°C, respectively. An ionization energy of 70 eV and a mass range of 50–250 u at 1 scan/s were used.

A Supelco (Gland, Switzerland) Visiprep SPE vacuum manifold was used for the clean-up procedure.

2.3. Sample collection

Atmospheric aerosols were collected in one of the main avenues of Barcelona, 10 m above

street level. This area has a high volume of traffic. The samples were collected on thermally treated filters (300°C for 2 h) using a Sierra Misco Model 650 high-volume sampler and a Whatman EPM-2000 20.3 × 25.4 cm glass-fibre filter-paper (Whatman International, Maidstone, UK). After collection, the filters were stored in at −30°C in the dark until analysis.

2.4. Preparation of samples

The filters were cut into four pieces and each piece was subjected to ultrasonic extraction, first with dichloromethane and then with methanol. The extracts were mixed and after filtration they were concentrated to dryness by rotary evaporation. The residue was redissolved in dichloromethane (50 ml) and subjected to liquid–liquid partitioning with 0.1 *M* NaOH (3 × 10 ml). Dichloromethane in the aqueous phase was removed using rotary evaporation. Finally, the aqueous extract was acidified to pH 4 and used for the solid-phase extraction as follows.

First, the C_{18} cartridge was cleaned with 10 ml of acetonitrile, 10 ml of acetone, 10 ml of methanol and 10 ml of water (pH 4) consecutively. The aqueous extract was introduced at a flow-rate of 4–5 ml/min and the column was washed with 10 ml of water (pH 4). After drying, the hydroxy-PAHs were eluted with 2 ml of methanol. The solvent was evaporated under nitrogen and the residue was dissolved in 500 μl of dichloromethane for GC–MS analysis or 1 ml of mobile phase and 10 μl of 2,4-dibromophenol (40 μg/ml) as internal standard for HPLC analysis.

3. Results and discussion

A standard solution (1 mg/ml) of seven hydroxy-PAHs (5-HI, 2-H-1,4-NQ, 5-IOH, 5,8-DH-1,4-NQ, 2-H-9-FLO, 9-HF and 2-N-1-N) was used and 2,4-dibromophenol was added as an internal standard. These compounds may be formed in the atmosphere due to reactions between PAHs and OH radicals and some have been detected in atmospheric aerosols [18].

Different binary phases of methanol and 40 m*M* phosphoric acid–potassium dihydrogenphosphate buffer (pH 3) from 70:30 to 45:55 were tested. The buffer solution provides the pH, the conductivity and the ionic strength needed for the electrochemical reactions. 5,8-DH-1,4-NQ gave tailing peaks, probably owing to the interactions with the silica support. The tailing effect was reduced by the addition of 3.5% acetic acid to the mobile phase. In addition, a decrease in methanol content produced an increase in the analysis time, and resolutions were improved except for 9-HF and 2-N-1-N. Further, the elution order of 2-H-9-FLO and 5,8-DH-1,4-NQ changed when the percentage of methanol in the mobile phase decreased. The optimum separation for all substances was obtained with the mobile phase methanol–phosphoric acid–sodium dihydrogenphosphate buffer (40 m*M*, pH 3) (50:50) plus 7% acetic acid (Fig. 2).

The optimum working potential was obtained from the hydrodynamic voltammograms of the compounds under the separation conditions previously established. Fig. 3 shows the hydrodynamic voltammograms. The working potential chosen was the potential that gave the best response for all the compounds. Good values were obtained at +550 mV for 5-HI, 5-IOH, 5,8-DH-1,4-NQ, 2-H-9-FLO and 9-HF, but at this potential 2-H-1,4-NQ, 2-N-1-N and the internal standard (2,4-dibromophenol) gave no response. High responses were obtained at potentials near +800 mV for all the compounds. At more positive potentials, increases in both the background noise and the residual current occurred owing to the oxidation of the mobile phase. Therefore, +800 mV was chosen as the optimum potential, although a slight decrease in the responses of 5,8-DH-1,4-NQ, 9-HF and 2-H-9-FLO was observed. Adsorption of the subproducts from the electrochemical reactions or polymerization on the electrode surface may be the cause of the decrease in the relative intensities.

Calibration for hydroxy-PAHs using peak areas in the range 0.2–20 μg/ml was carried out

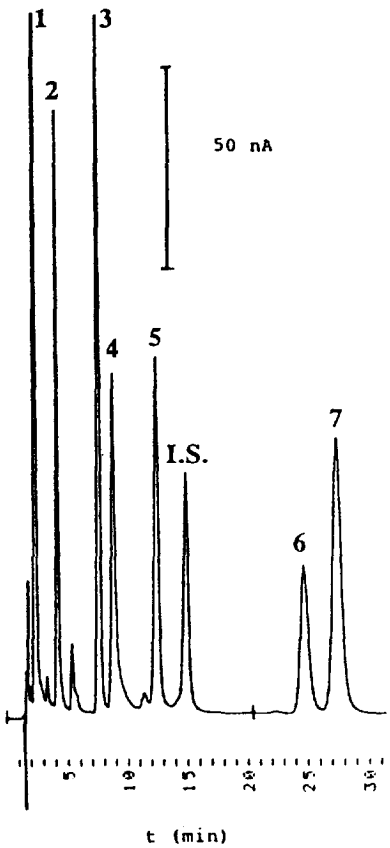


Fig. 2. Chromatogram of hydroxy-PAHs (20 μl, 0.4 μg/ml). Mobile phase: methanol–phosphoric acid–sodium dihydrogenphosphate buffer (40 mM, pH 3) (50:50) plus 3.5% acetic acid in buffer solution. Peaks: 1 = 5-HI; 2 = 2-H-1,4-NQ; 3 = 5-IOH; 4 = 5,8-DH-1,4-NQ; 5 = 2-H-9-FLO; 6 = 9-HF; 7 = 2-N-1-N.

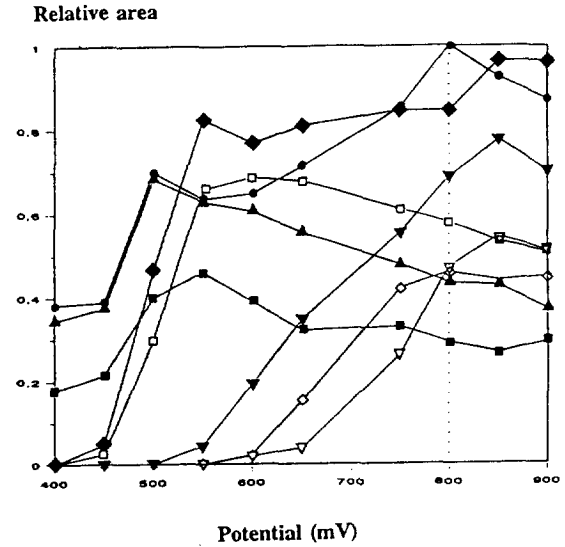


Fig. 3. Hydrodynamic voltammograms: ▲ = 5-HI; ● = 2-H-1,4-NQ; ▽ = 5-IOH; ◇ = 5,8-DH-1,4-NQ; ▼ = 2-H-9-FLO; ◆ = internal standard; □ = 9-HF; ■ = 2-N-1-N.

under the optimum separation conditions for each compound. The correlation coefficients of the calibration functions for eight concentration levels from 0.01 to 1 μg/ml (two replicates) were better than 0.9994 for all the hydroxy-PAHs. Five replicate determinations of 8 ng (0.4 μg/ml solution) of each hydroxy-PAH in the mobile phase were carried out under the optimum conditions to determine the precision of the analysis. The relative standard deviations (R.S.D.) were in the range 0.9–4.5%.

The detection limits of the HPLC–ED system were determined as three times the signal-to-noise ratio for peak areas using standard solutions. For the early peaks, 5-HI, 5-IOH and 2-H-1,4-NQ, the detection limits were low, 30, 105 and 90 pg, respectively. The higher value obtained for 5,8-DH-1,4-NQ, 220 pg, may be related to its low response at the working potential (+800 mV) (Fig. 3). The compounds that eluted with relatively long retention times gave high detection limits of 200 pg for 2-H-9-FLO, 240 pg for 2-N-1-N and 300 pg for 9-HF.

The method presented was mainly developed to determine PAH derivatives in samples of environmental concern, and hydroxy-PAHs were determined in various atmospheric aerosols. Fig. 4A shows a typical chromatogram of a winter atmospheric aerosol and Fig. 4B shows the chromatogram obtained when the sample was spiked with 6 μg of hydroxy-PAHs per gram of particulate matter. These chromatograms indicate that 2-nitro-1-naphthol can be present in the extract. Minor peaks at the retention times of 2-hydroxy-9-fluorenone and 5-hydroxyindane appeared, suggesting that these compounds could be present in the extract. The concentration of 2-N-1-N in the sample was calculated by HPLC–

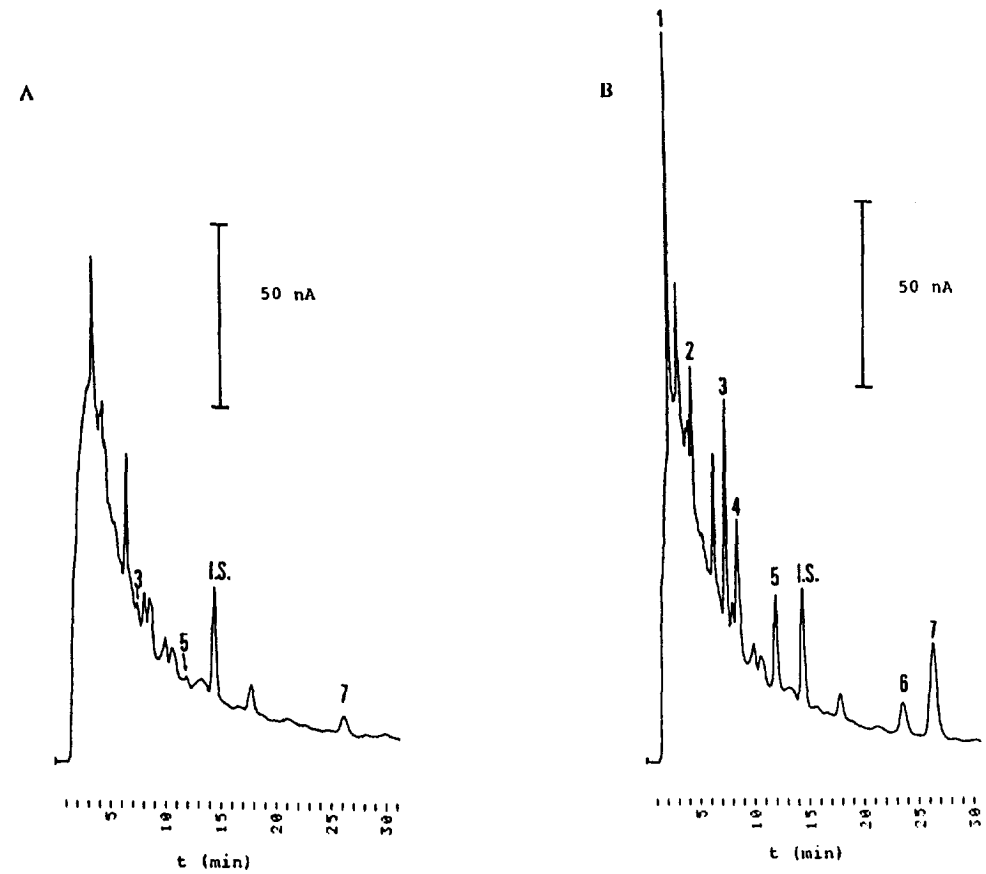


Fig. 4. HPLC of (A) atmospheric aerosol sample and (B) spiked atmospheric aerosol sample. Chromatographic conditions as in Fig. 2. Peaks: 1 = 5-HI; 2 = 2-H-1,4-NQ; 3 = 5-IOH; 4 = 5,8-DH-1,4-NQ; 5 = 2-H-9-FLO; 6 = 9-HF; 7 = 2-N-1-N.

ED using 2,4-dibromophenol as internal standard, and was found to be 0.21 ng/m^3 in air and 19 μg/g in particulate matter. The concentrations of the 5-IOH and 2-H-9-FLO, tentatively identified by HPLC–ED, would be of the order of the detection limits for real samples, which are always higher than those obtained for standard solutions. For hydroxy-PAHs in aerosol samples, the detection limits calculated from the spiked sample were 0.8–2.5 ng injected into the chromatograph, 5–30 times higher than those obtained from standard solutions. The highest differences were observed for the early-eluted peaks owing to the baseline tail. Taking into account the recovery of the preconcentration step, these values mean that the detection limits of these compounds in real samples would be of the order of 0.03–0.2 ng/m^3, depending on the compound.

In order to confirm the presence of hydroxy-PAHs in the aerosol samples, the extracts dissolved in dichloromenthane were injected into a GC–MS system. Only 2-N-1-N was identified in the winter sample; its spectrum and the total ion chromatogram are given in Fig. 5. The concentrations of 2-H-9-FLO and 5-IOH, of the order of 0.05 ng/m^3 according to their detection limits by HPLC–ED, were not high enough for their identification by GC–MS.

Although there are few data in the literature

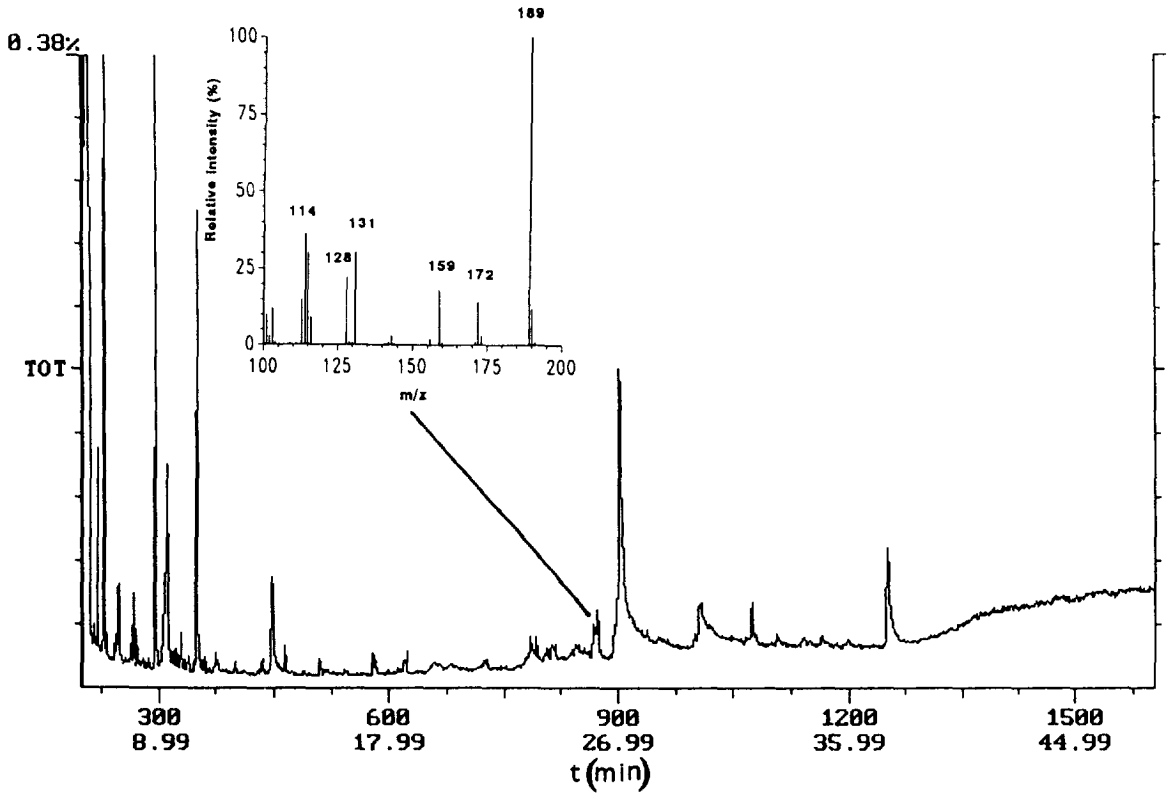


Fig. 5. Total ion gas chromatogram of an atmospheric aerosol sample and mass spectrum of the compound indentified (2-N-1-N).

about hydroxy-PAHs in atmospheric aerosols, our results agree with the values found by Nishioka et al. [13], who reported concentrations for hydroxylated nitro polycyclic aromatic compounds in urban air particulate extracts between 0.01 and 0.6 ng/m^3.

4. Conclusions

The use of liquid chromatography with electrochemical detection for the determination of hydroxy-PAHs has been assessed. Separation and determination conditions to provide detection at the subnanogram level were established. The selectivity, linearity and sensitivity of the method were studied. The method was applied to the determination of hydroxy-PAHs in atmospheric aerosol samples.

Acknowledgement

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