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The determination of 3-nitrophenol and some other aromatic impurities in 4-nitrophenol by reversed phase HPLC with peak suppression diode array detection

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Abstract

In this work the peak suppression technique is used for the determination of 3-nitrophenol and some other aromatic impurities in 4-nitrophenol by reversed phase HPLC with diode array detection. Taking into account the differences between the absorption spectra of the two compounds, two wavelengths were selected in order to obtain the maximum difference between the spectral contribution for 3-nitrophenol and to maintain a small, similar spectral contribution for 4-nitrophenol (the main compound). Then we used the wavelength corresponding to a small spectral contribution of 3-nitrophenol as the reference wavelength. It was shown that taking $\lambda_{an} = 266$ nm and $\lambda_{ref} = 364$ nm, a broad elution peak of 4-nitrophenol was suppressed deconvoluting the peak of 3-nitrophenol. Moreover, quantitation of 3-nitrophenol was achieved without chemometric tools. Under the proposed conditions the detection limits for 3-nitrophenol and other common impurities of 4-nitrophenol used in the pharmaceutical industry (4-chlorophenol, 4-nitrophenol, 1-chloro-2-nitrobenzene, 1-chloro-4-nitrobenzene, 4,4′-bisfenilether, and 4,4′-dichloroazobenzene) were not significantly affected as compared with respective detection limits evaluated in the absence of 4-nitrophenol and using standard detection conditions ($\lambda_{an} = 280$ nm and $\lambda_{ref} = 420$ nm).

Keywords: 3-Nitrophenol; Impurities; Reversed phase HPLC; Diode array UV–Vis detector; Differential spectrophotometry

1. Introduction

4-Nitrophenol is a substrate needed in the pharmaceutical industry for acetaminophen (paracetamol) synthesis and, thus, its purity is of vital importance. Technical 4-nitrophenol is contaminated with 3-nitrophenol and other aromatic compounds, which are by-products of synthesis or

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degradation products. For industrial applications rapid and simple procedures are preferred, without or with minimum sample treatment and avoiding complicated gradient programmes [1]. Thus, chemometric techniques are frequently used to extract analytical information from overlapping peaks instead of time- and labour-consuming procedures needed to achieve baseline resolution [2]. Several techniques based on spectral differences of the solutes have been described for discrimination of overlapped chromatographic peaks obtained with diode array detection [3–14], one of them being the peak suppression technique [3–9].

Taking advantage of the differences in absorption spectra of 3- and 4-nitrophenol [15–17] we propose here the non-chemometric approach to achieve suppression of the 4-nitrophenol peak on a reversed phase chromatogram of the two compounds. Following the principle of the peak suppression technique the two wavelengths were selected assuring the maximum difference between the spectral contribution for 3-nitrophenol, but keeping at a similar level the small spectral contribution for 4-nitrophenol. Then, instead of obtaining the chromatograms at the selected wavelengths, we used one of them (corresponding to a small spectral contribution of 3-nitrophenol) as the reference wavelength.

2. Experimental

2.1. Apparatus

A Hewlett–Packard (Waldbronn, Germany) series 1050 high-performance liquid chromatograph with a multiple wavelength spectrophotometric detector and Chem-Station was used. A Shandon Hypersil ODS column (5 μm, 250 × 4.6 mm) was used at room temperature.

2.2. Reagents

The solvents were of HPLC-grade quality and all other chemicals were of analytical-grade.

2-Nitrophenol, 3-nitrophenol, 4-nitrophenol, 4-chlorophenol, 1-chloro-2-nitrobenzene and 1-chloro-4-nitrobenzene were from Aldrich. 4,4′-Bisfenilether was from TCI (Japan), 4,4′-dichloroazobenzene was synthetized from 4-chloronitrobenzene (Aldrich) as described elsewhere [18] and crystalized from ethanol.

A stock standard solution of 4-nitrophenol was prepared by dissolving 2500 mg of the reagent (Aldrich) in 25 ml acetonitrile. Stock standard solutions of other compounds were prepared similarly and contained 25 mg of the compound in 25 ml acetonitrile.

Aqueous phosphoric acid solution (2.0 mol l^{-1}) was prepared from concentrated acid (Sigma, ACS reagent) and purified on the Sep-Pak® Plus C18 cartridge (Waters). The 20 mmol l^{-1} solution was obtained by appropriate dilution with deionized water.

Acetonitrile was from Fisher Scientific.

Deionized water (Labconco) was used throughout.

2.3. Procedures

Chromatographic separation of 4-nitrophenol and seven possible impurities was achieved on the ODS column with mobile phase containing 20 mmol l^{-1} aqueous phosphoric acid (A) and acetonitrile (B) at a flow rate of 1.2 ml min^{-1}. The injection volume was 20 μl and the following gradient was used: 0–3.5 min 40% B, 3.5–8.0 min 90% B, 8.0–10.0 min 95% B, 10.0–14.0 min 95% B, and 14.0–14.5 min 40% B. Diode array spectrophotometric detection was carried out at 266 ± 8 nm with the reference wavelength at 364 ± 8 nm. The peak area was taken as the measurement mode.

The calibration solutions for 3-nitrophenol (0–0.50 μg ml^{-1}) were prepared by diluting the stock standard solution with 20 mmol l^{-1} phosphoric acid–acetonitrile (6:4). For the standard addition technique four sets of solutions were prepared containing 5, 10, 15, and 20 mg ml^{-1} 4-nitrophenol. The additions of 3-nitrophenol in each set corresponded to 10, 25, 50, 75, and 100 μg of the compound per gram of 4-nitrophenol (ppm of impurity). The peak area was evaluated using tangent skim integration. The detection limit for 3-nitrophenol was evaluated by the method based

Table 1
Retention time of 4-nitrophenol and of its possible impurities together with detection limits of these compounds evaluated as 3 S.D.s of baseline noise (except for the 3-nitrophenol) using different detection conditions and in the presence of 10 mg ml^{-1} 4-nitrophenol

Compound	t_{ret}	Detection limits (ng ml^{-1})	
		$\lambda_{an} = 280$ nm; $\lambda_{ref} = 420$ nm	$\lambda_{an} = 266$ nm; $\lambda_{ref} = 364$ nm
4-Nitrophenol	5.07	–	–
3-Nitrophenol	5.47	–	61
4-Chlorophenol	6.85	70	42
2-Nitrphenol	7.36	17	15
1-Chloro-2-nitrobenzene	8.65	6	8
1-Chloro-4-nitrobenzene	8.98	4	3
4,4′-Bisfenilether	9.75	8	9
4,4′-Dichloroazobenzene	13.38	28	39

on regression S.D. of instrument signal to analyte concentration [19].

Calibration of 2-nitrophenol, 4-chlorophenol, 1-chloro-2-nitrobenzene, 1-chloro-4-nitrobenzene, 4,4′-bisfenilether and 4,4′-dichloroazobenzene was carried out in 10 mg ml^{-1} 4-nitrophenol standard solution taking concentrations of each compound in the range corresponding to 10–100 μg per gram of 4-nitrophenol (ppm of impurity). The chromatograms were registered using the two sets of wavelengths: (1) one set proposed in this work (detection: 266 ± 8 nm, reference: 364 ± 8 nm), and (2) one set commonly used for diode array detection of aromatic compounds (detection: 280 ± 20 nm, reference: 420 ± 40 nm). The peak area was measured using V/V integration. The detection limits for the above mentioned compounds were evaluated as 3 S.D.s of baseline noise.

3. Results and discussion

The common impurities of 4-nitrophenol used in the pharmaceutical industry include 3-nitrophenol, 4-chlorophenol, 2-nitrophenol, 1-chloro-2-nitrobenzene, 1-chloro-4-nitrobenzene, 4,4′-bisfenilether and 4,4′-dichloroazobenzene. Baseline separation of these compounds was achieved (chromatographic conditions given in Section 2.3) and the respective retention times are given in Table 1. As can be observed in Table 1, the resolution obtained for 4-nitrophenol ($t_{ret} = 5.07$ min) and 3-nitrophenol ($t_{ret} = 5.47$ min) enables quantitation of the two compounds if they are present at similar and low concentrations. However, in the presence of a great excess of 4-nitrophenol in the sample, traces of 3-nitrophenol would be eluted in the tail of the first peak.

In order to suppress the peak 4-nitrophenol (the main compound) the principle of the peak suppression technique was applied. The original

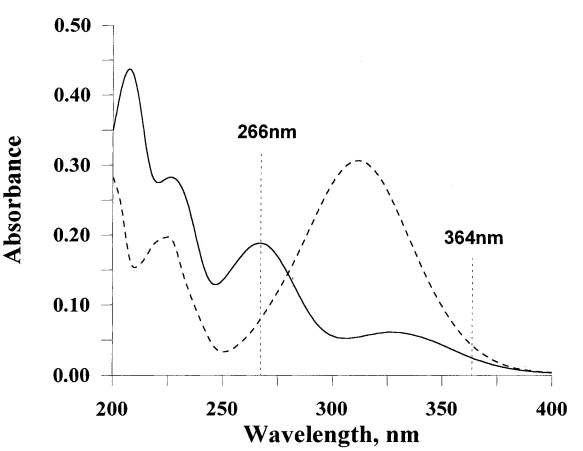

Fig. 1. Absorption spectra of (---) 4-nitrophenol, 5 μg ml^{-1} and (—) 3-nitrophenol, 5 μg ml^{-1} in 20 mmol l^{-1} phosphoric acid–acetonitrile (6:4).

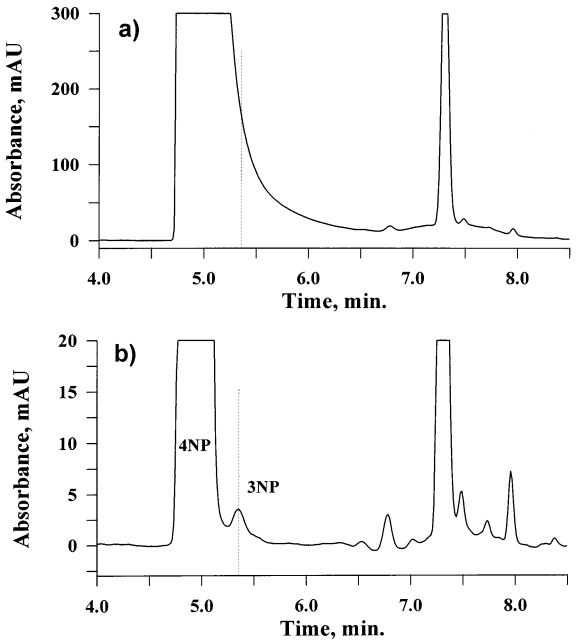

Fig. 2. Chromatogram of 4-nitrophenol (4NP), 20 mg ml^{-1} and 3-nitrophenol (3NP), 0.2 μg ml^{-1} using different detection conditions: (a) $\lambda_{an} = 280$ nm, $\lambda_{ref} = 420$ nm; and (b) $\lambda_{an} = 266$ nm, $\lambda_{ref} = 364$ nm.

idea of this work was to use one of the selected wavelengths as the reference, thus assuring different detection sensitivity for 4- and 3-nitrophenol on one chromatogram and without the necessity of chemometrics. It can be observed in Fig. 1 where the absorption spectra of the two compounds are presented, that using 266 nm as the analytical wavelength and the reference wavelength in the region of 360 nm, low sensitivity should be obtained for 4-nitrophenol and high sensitivity for 3-nitrophenol. Using 266 nm for detection the effect of the reference wavelength (360–420 nm) on the peak area of 3- and 4-nitrophenol was studied. The reference wavelength of 364 nm was selected as the most suitable: the suppression of the peak area of 4-nitrophenol was to about 2%, while the peak area of 3-nitrophenol was suppressed only to about 75% as referred to the respective signals obtained with the reference at 420 nm (100%). In Fig. 2 typical chromatograms of the solution containing 4-nitrophenol (20 mg ml^{-1}) and 3-nitrophenol (0.2 μg ml^{-1}) are presented, which were obtained using different detection conditions. It is clear that with the detection conditions proposed here (Fig. 2b) the main component peak was suppressed and the quantitation of 3-nitrophenol became possible as compared with the detection at 280 nm and reference at 420 nm (Fig. 2a).

Using the proposed detection conditions ($\lambda_{an} =$ 266 nm, $\lambda_{ref} = 364$ nm), the calibration of 3-nitrophenol was carried out in the absence of 4-nitrophenol and using two measurement modes, namely peak height and peak area. Then, using these same conditions, four linear regression functions for 3-nitrophenol standard additions were evaluated in the presence of different concentrations of 4-nitrophenol (details given in Section 2.3). It was observed that, up to 10 mg ml^{-1} of 4-nitrophenol and using the peak area measurement mode with tangent skim integration mode, the slope of the linear regression function for 3-nitrophenol was not significantly changed as compared with the slope obtained in the absence of 4-nitrophenol (the difference no higher than 5%). For higher concentrations of the major compound (15 and 20 mg ml^{-1}), the slope of the linear regression function for 3-nitrophenol decreased respectively to 85 and to 75% as referred to the slope obtained in the absence of 4-nitrophenol. This effect was probably caused by negative errors committed using integration of the signals obtained under 'differential' conditions. It should be mentioned that, using the peak height measurement mode, lower sensitivity (slope of linear regression function) was observed which can be ascribed to the lower efficiency performance of the column when 0.1–0.4 mg of 4-nitrophenol was introduced (column overload). Resuming, the results obtained indicate that, using the detection conditions proposed here and the peak area measurement mode with tangent skim integration, quantitation of 3-nitrophenol is not interfered with by the presence of 4-nitrophenol up to a concentration of 10 mg ml^{-1} in the solution. The detection limits for 3-nitrophenol in the presence of 5 and 10 mg ml^{-1} 4-nitrophenol were evaluated by the method based on regression S.D. of instrument signal to analyte concentration [19]

(using tangent skim integration the detection limit can not be evaluated as 3 S.D.s of baseline noise). The values obtained were respectively: 46 and 61 ng ml^{-1}, which correspond to 9.2 and 6.1 ppm of impurity, while in the absence of 4-nitrophenol, the detection limit for 3-nitrophenol was 28 ng ml^{-1}.

Finally, the effect of the proposed conditions on the detection power for other possible impurities of 4-nitrophenol was studied. The calibration solutions of 4-chlorophenol, 2-nitrophenol, 1-chloro-2-nitrobenzene, 1-chloro-4-nitrobenzene, 4,4′-bisfenilether and 4,4′-dichloroazobenzene were prepared with addition of 4-nitrophenol (10 mg ml^{-1}) and chromatograms were obtained as described in Section 2.3. The detection limits of the above mentioned compounds evaluated using the analytical wavelength 280 nm and the reference wavelength 420 nm are compared in Table 1 with the detection limits evaluated using the proposed detection conditions. From Table 1 it can be observed that, using our conditions, lower values were obtained only for 4-chlorophenol. This means that, the elution peak of this compound ($t_{ret} = 6.85$ min) registered with the common UV–Vis detection conditions was affected by the broad peak of 4-nitrophenol ($t_{ret} = 5.07$ min, concentration 10 mg ml^{-1}) and the use of detection conditions suppressing the peak of 4-nitrophenol enabled to us reduce such interferences. As expected, for 2-nitrophenol ($t_{ret} = 7.36$ min), 1-chloro-2-nitrobenzene ($t_{ret} = 8.65$ min), 1-chloro-4-nitrobenzene ($t_{ret} = 8.98$ min) and 4,4′-bisfenilether ($t_{ret} = 9.75$ min) the detection limits obtained using different detection conditions were similar. In the case of 4,4′-dichloroazobenzene ($t_{ret} = 13.38$ min) the detection limit can be improved using analytical wavelength 330 nm ($\lambda_{ref} = 420$ nm) corresponding to the maximum on its absorption spectrum (detection limit = 12 ng ml^{-1}).

4. Conclusions

In this work, the peak suppression technique was used for the determination of 3-nitrophenol impurities in 4-nitrophenol by reversed phase HPLC. It was shown that appropriate selection of the analytical and the reference wavelengths helps to suppress a broad elution peak of the main compound enabling deconvolution of the minor compound peak and its quantitation without a chemometric approach. Using analytical wavelength 266 nm with the reference at 364 nm, the loss of sensitivity for 3-nitrophenol determination was only about 5% in the presence of 10 mg ml^{-1} of 4-nitrophenol as referred to the sensitivity obtained in the absence of this compound. The detection limit achieved was 61 ng ml^{-1}, which corresponds to 6.1 ppm of impurity. As to the detection power for other possible aromatic impurities of 4-nitrophenol, using modified conditions, an improvement was observed for 4-chlorophenol.

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