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Short communication

Determination of 4-aminophenol impurities in multicomponent analgesic preparations by HPLC with amperometric detection

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Abstract

A method for the determination of 4-aminophenol, the main impurity of paracetamol, by high-performance liquid chromatographic (HPLC) method with amperometric detection has been developed. The analysis was performed in an isocratic mode on a reversed phase Luna column 5 μm C-18 (100 × 4.6 mm). A mobile phase (0.05 mol l^{-1} LiCl solution containing 18% methanol adjusted to pH 4.0 with orthophosphoric acid) was suitable for the separation and determination of 4-APh. Chromatograms were recorded for 250 s by means of an amperometric detector at a potential of +325 mV of the glassy carbon electrode versus the reference electrode Ag/AgCl. The proposed liquid chromatographic method was successfully applied to the analysis of commercially available multicomponent dosage forms. The sensitivity of the detection for 4-aminophenol was 1 ng ml^{-1} for substance and 4 ng ml^{-1} for tablets or capsules. The method developed in this study is sensitive and selective and can be applied for routine studies of pharmaceuticals in the form of tablets or capsules.

Keywords: 4-Aminophenol; Paracetamol; HPLC method with amperometric detection, determination; Pharmaceutical formulations

1. Introduction

Paracetamol is a drug of analgesic, antipyretic and antiflogistic activity widely applied in therapeutics. This compound is a component of analgesic preparations, both the single-ingredient and multicomponent ones. Except paracetamol, the following compounds belong also to this group of medical products: acetylsalicylic acid, pseudoephedrine, dextromethorphan, guaiaphenasin, chlorpheniramine, codeine, mepiramine, propyphenazone, vitamin C and caffeine. The main impurity, which may be present in the preparations containing paracetamol, is 4-aminophenol (4-APh). This compound may originate during the synthesis of paracetamol or may be formed during

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the storage of preparations. 4-APh is a substance of moderately low toxicity. During studies with the use of animals, nephrotoxicity of 4-APh was observed and also its capability of causing methemoglobinemy. Studies for the presence of this impurity in paracetamol are provided by BP 1998, USP 24, DAB 10, Ph. Eur. 2000 and in the various dosage forms of the drug by BP 1998 and numerous specifications of manufacturers.

In the literature, there is a number of papers dealing with the determination of 4-aminophenol in substance, pharmaceutical preparations and biological materials as it was already mentioned in the first part of this study [1]. 4-Aminopehnol may also take part in enzymatic reactions. Zhang et al. [2] used electrochemical and spectral methods to study oxidation reaction of 4-APh by hydrogen peroxide in the presence of horseradish peroxidase as a catalyst. This reaction was used to enzyme-linked immunoassay, in which thyroxine [3], alpha-fetoprotein [4] and cucumber mosaic virus [5] as well as southern bean mosaic virus [6] were determined. In the latter studies, electrochemical (amperometric or voltammetric) detection was used. On the other hand, Gao et al. [7] studied the formation of 4-APh complexes with beta-cyclodextrin by fluorescence spectroscopy and square wave voltammetry.Recently, FIA [8] and NMR [9] methods of 4-aminophenol determination were described. Karousos and Reddy [10] examined this compound using quartz microbalance sensor.

In part one [1] of this study, we investigated the electrode behavior of 4-aminophenol in a paracetamol substance using the cyclic and differential pulse voltammetric methods in various non-buffered and buffered solutions at glassy carbon and gold electrodes. By means of the high-performance liquid chromatographic (HPLC)-ED method the dependence of current intensities of 4-APh peaks on the potential in the range 0–600 mV, in the pH range 2–5, the ionic strength of the mobile phase ranging from 0.01 to 0.20 mol l^{-1} LiCl, has been studied. We elaborated an HPLC method with amperometric detection for the determination of 4-APh in a paracetamol substance (Aldrich) and in single-ingredient tablets containing 500 mg of paracetamol per tablet. In these HPLC-ED studies we used a glassy carbon electrode as the amperometric detector. It was found that a 0.05 mol l^{-1} LiCl solution, containing 18% of methanol of pH 4.0 adjusted with orthophosphoric acid is suitable for the separation of 4-APh and paracetamol one from the other and from a pharmaceutical excipients.

4-Aminophenol is the primary degradation product of paracetamol which is limited at a low level (50 ppm or 0.005% w/w) in the drug substance by the European, United States, British and Germans Pharmacopoeias, employing a manual colorimetric limit test. This low level ensures paracetamol drug safety as 4-APh is reported to have nephrotoxicity and teratogenic effect. The 4-APh limit is widened to 1000 ppm or 0.1% w/w for the tablet product monographs, which quote the use of a less sensitive automated HPLC method. So when the lower drug substance specification limit is applied, the HPLC method is not suitable at this low level due to matrix interference. Therefore, we decided to develop a rapid and sensitive HPLC-ED method for the determination of 4-APh impurities, at low levels, in multicomponent analgesic preparations with paracetamol. The developed HPLC method should makes it possible to separate 4-APh from the matrix connected with the dosage form while the sensitivity and selectivity of the electrochemical detection should enable to determine a very low concentration of 4-aminophenol (lower than 50 ppm, equivalent to 25 μg 4 APh in tablet containing 500 mg of paracetamol) in pharmaceutical preparations. The developed HPLC-ED method should make it possible to determine the real content of 4-APh, which is considerably below the detection limit of the official analytical methods.

2. Experimental

2.1. Apparatus and conditions

The following apparatus were used throughout this work: a μAutolab voltammeter (Eco Chemie, Utrecht) controlled by a computer with an accompanying software GPES (General Purpose Electrochemical System Version 4.8); a liquid

chromatograph, type LC-10AP and a degasser DGU-14A (Shimadzu), an electrochemical detector Flexell of active volume 0.5 μl with a glassy carbon electrode (working electrode), a silver–silver chloride electrode (Ag/AgCl, reference electrode), a steel wire (auxiliary electrode), an SSI Pulse Damper (Antec, Leyden).

The analysis was performed in isocratic mode on a Luna 5 μm C18 analytical column (100 × 4.6 mm, I.D., Phenomenex, Torrance, CA). The mobile phase (0.05 mol l^{-1} lithium chloride solution containing 18% of methanol adjusted to pH 4.0 with 85% orthophosphoric acid) was filtered through filters (0.45 μm, Whatman, USA) and degassed with a Sonifier 250/450 (Branson Ultrasonics Corporation, USA) before use. The column was maintained at 30 °C in a column block heater. A flow rate was set at 1 ml min^{-1} and sample injections were typically 20 μl.

Oxygen was removed from the system by passing argon for 15 min.

2.2. Materials studied

A 4-aminophenol-substance (Aldrich) and the following investigated preparations (in the brackets, the content of paracetamol and average weight of the preparation are given): antidol coated tablets from Argon S.A. (300 mg, 0.3457 g); cefalgin tablets from Polfa, Pabianice (250 mg, 0.6970 g); codespan tablets from Polpharma S.A. (500 mg, 0.6558 g); dolores tablets from Curtis Healthcare (500 mg, 0.6965 g); gripex coated tablets from US Pharmacia (325 mg, 0.4628 g); grippostad C capsules from Stada (200 mg, 0.4776 g); grypolek tablets from Kato Laboratories Inc. (325 mg, 0.8910 g); hedalgan tablets from Polpharma S.A. (400 mg, 0.5474 g); neopyrin tablets from Biofarm (100 mg, 0.6452 g); pabitan tablets from Polfa Pabianice (500 mg, 0.6910 g); promiss coated tablets from Kato (500 mg, 0.6930 g); saridon tablets from Hoffman-La Roche (250 mg, 0.6490 g); tabcin capsules from Bayer (250 mg, 1.3519 g); talvosilen tablets from bene-Arzneimittel GmbH (500 mg, 0.6147 g); tomapyrin tablets from Boehringer Ingelheim (200 mg, 0.6002 g).

2.3. Reagents

Orthophosphoric acid from Riedel-de-Haën, methanol from LAB-SCAN, lithium chloride from BDH—all of them of purity suitable for AAS and HPLC; doubly distilled water additionally purified in the Nanopure Deionization System (Barnstead) were used throughout.

2.3.1. Mobile phase

0.05 mol l^{-1} lithium chloride solution containing 18% of methanol adjusted to pH 4.0 with 85% orthophosphoric acid.

Standard solution of 4-aminophenol at a concentration of about 100 ng ml^{-1} was daily prepared by dissolving about 10 mg of 4-APh in the mobile phase in a 50 ml volumetric flask. Then, 0.5 ml of the prepared solution was added to a 50 ml volumetric flask and made up to the mark with the mobile phase. Standard solution of paracetamol of concentration about 7 mg ml^{-1} was daily prepared by dissolving about 175 mg of paracetamol in the mobile phase in a 25 ml volumetric flask. All solutions were stored in the dark at 4 °C when not in use.

2.4. Preparation of samples and determination of 4-aminophenol in pharmaceutical formulations

An average weight of ten tablets was determined. The tablets were powdered and an accurate weight of this material or the content of capsules, equivalent to 0.5 g of paracetamol was transferred into 100 ml volumetric flasks. Then 50 ml of the mobile phase was added and the solution was mechanically shaken for 20 min. The flasks were filled with mobile phase. Thus, prepared solutions were filtered through filters (0.45 μm, Whatman, USA) and the first 5 ml of the filtrate was removed. From the filtrates 7 ml of gripex, tabcin and talvosilen, 8 ml of pabitan or 9 ml of grypolek were withdrawn and made up to 10 ml volume with the mobile phase and mixed well. Then 20 μl of the prepared solutions were introduced into the column and chromatograms were recorded for 250 s by means of an amperometric detector at a potential of +325 mV of the glassy carbon electrode versus the reference electrode Ag/AgCl.

The current intensity of the 4-aminophenol was measured for the retention time of about 71 s, and that of paracetamol for 185 s.

There are more than 20 different pharmaceutical excipients present in tablets or capsules of paracetamol. They were not electrochemically active and most of them were insoluble in the mobile phase. It was confirmed by the recovery tests (known amounts of 4-APh were added to an inert tablet formulations and the mixtures passed through the procedure) that pharmaceutical excipients did not influence the determination of 4-aminophenol. The content of 4-APh in tablets and capsules was assayed by the double standard-addition method.

3. Results and discussion

The developed HPLC method with amperometric detection [1] was applied to the determination of 4-aminophenol in multicomponent analgesic preparations containing paracetamol as one of the active substances.

In order to obtain optimal chromatographic separation, different mobile phases and columns were evaluated. The resolution and separation of all electroactive substances would be more sufficient with the use of a gradient system, but this procedure is not suitable for amperometric detection. In this study several parameters were examined in order to optimize amperometric detection of 4-APh. The +325 mV potential has been chosen as optimal for this study. At this potential one stable, well shaped peak of 4-APh and a relatively small peak of paracetamol was recorded. It was found that a 0.05 mol l^{-1} LiCl solution containing 18% of methanol adjusted to pH 4.0 with orthophosphoric acid is suitable for the separation of 4-APh and paracetamol, also from other active substances or from pharmaceutical excipients present in multicomponent analgesic preparations. In the first part of this work [1], the dependence of the intensity of anodic 4-aminophenol peak on its concentration (1–2000 ng ml^{-1}) in the presence of a large excess (80 000-fold) of paracetamol was studied. It was found that the obtained calibration curve was of linear character in the whole concentration range studied (from 1 to 55 ng ml^{-1} $y = 0.755x + 7.823$ $r = 0.998$, from 55 to 2000 ng ml^{-1} $y = 0.412x + 17.549$ $r = 0.998$). The detection limit of 4-APh for the substance was 1 ng ml^{-1} and for the tablets and capsules 4 ng ml^{-1}. Quantitative parameters of 4-aminophenol determination are presented in Table 1.

In the obtained chromatograms (Fig. 1) beside the 4-APh peak and that of paracetamol, an additional peak was observed (retention time 134 s), which originates from impurity other than 4-APh. An attempt was undertaken to identify this peak. It was found that it is neither 4-chloroacetanilide, a reagent used in the synthesis of paracetamol and described by pharmacopoeias, nor pharmaceutical excipients present in tablets. Probably it is another intermediate, formed in the process of paracetamol synthesis.

The stability of the 4-APh standard and sample preparations in mobile phase was demonstrated by analysis of solutions stored at ambient temperature and at 4 °C, away from direct sunlight, over a period of 24 h. It is recommended that the solutions od 4-APh and sample preparations are stored at 4 °C.

Using the developed HPLC-ED method, the content of 4-APh was determined in the following multicomponent analgesic preparations (Fig. 1): antidol, cefalgin, codespan, dolores, gripex, grippostad, grypolek, hedalgan, neopyrin, pabitan, promiss, saridon, tabcin, tolvosilen, tomapyrin.

Table 1
Quantitative parameters of 4-aminophenol determination by HPLC-ED method

Parameter	
Concentration range (ng ml^{-1})	(1) 1–55; (2) 55–2000
$Y = aX + b$	(1) $0.755X + 7.823$; (2) $0.412X + 17.549$
Correlation coefficient	(1) 0.998; (2) 0.998
Standard error of the slope	(1) 0.001; (2) 0.002
Standard error of the intercept	(1) 0.002; (2) 0.002
Detection limit (ng ml^{-1})	1.0
Quantitation limit (ng ml^{-1})	3.5
Between-day R.S.D. (%)	0.99
Within-day R.S.D. (%)	0.49
Precision of determination; mean $\pm$ $tS/\sqrt{N}$ (taken 106.4 ng ml^{-1}, N = 10)	105.35 ± 0.36

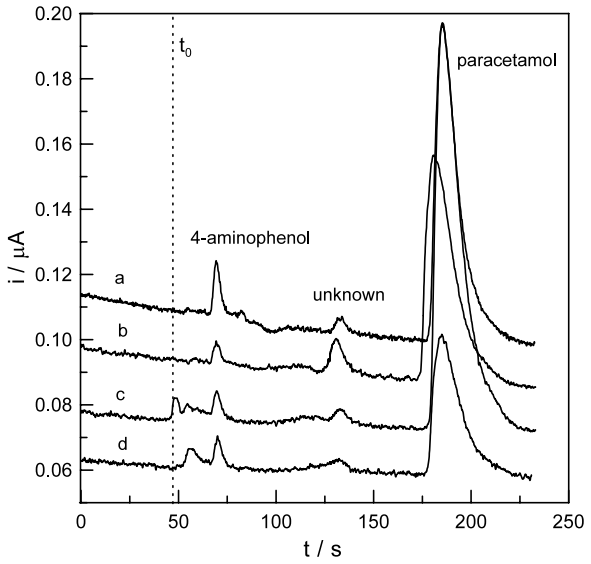

Fig. 1. Chromatograms recorded in 0.05 mol l^{-1} LiCl containing 18% of methanol of pH 4.0 for preparations: tabcin capsules, gripolek tablets, pabitan tablets and talvosilen tablets; flow rate 1 ml min^{-1}; amperometric detection, electrode potential +325 mV.

Statistical estimation of the results of the 4-APh determination in tablets and capsules is presented in Table 2. It was noted that in following preparations: antidol, cefalgin, codespan, hedalgan, dolores, neopyrin, promiss and saridon the 4-APh content was below the detection limit of the developed method.

It was found that 4-aminophenol can be determined in preparations containing paracetamol, pseudoephedrine, dextromethorphan, guaiophenasin, chlopheniramine, codeine, mepiramine, propyphenazone and caffeine. However, 4-APh cannot be determined when acetylsalicylic and ascorbic acids are present in the studied system.

In preparation grippostad C containing beside paracetamol also vitamin C, the applied experimental conditions and mobile phase did not enable the separation of 4-APh from vitamin C, both compounds showed approximate retention times. Thus, the separation of 4-APh under these conditions was impossible. To separate 4-APh from vitamin C an attempt has been made to perform a preliminary extraction of 4-APh with acetonitrile or ether. However, both 4-APh and ascorbic acid were extracted by the applied solvents so the intended separation was impossible. The change of the mobile phase composition and its pH value as well as the electrode potential did not allow to separate and determine 4-aminophenol.

In the case of tomapyrin it was found that the content of 4-APh in the filtrate from tablets was changing with time. A fast increase of the 4-APh content was observed, it was probably caused by a decomposition of paracetamol under the influence of large quantities (250 mg per tablet) of acetylsa-

Table 2
Statistical estimation of the results of 4-aminophenol determination in multicomponent analgesic preparations containing paracetamol by the HPLC-ED method (n = 9)

Preparation	S.D.	S.D. of arithmetic mean	R.S.D. (%)	$\bar{x} \pm t \times s\bar{x}$ (10^{-3}) Confidence level 95%
Gripex tablet "US Pharmacia" 325 mg of paracetamol	0.049	0.015	3.16	1.566 ± 0.035% of paracetamol content
Gripolek tablet "Kato Laboratories" 325 mg of paracetamol	0.005	0.002	3.04	0.187 ± 0.004% of paracetamol content
Talvosilen capsule "Bayer" 250 mg of paracetamol	0.009	0.003	2.55	0.386 ± 0.007% of paracetamol content
Pabitan tablet "Polfa Pabianice" 500 mg of paracetamol	0.01	0.003	3.38	0.303 ± 0.007% of paracetamol content
Tabcin capsules "Bayer" 250 mg of paracetamol	0.023	0.007	2.89	0.823 ± 0.018% of paracetamol content

licylic acid. This substance acidified the filtrate from tablets and caused an accelerated decomposition of paracetamol leading to an increase in the content of 4-aminophenol.

Polish Pharmacopoeia V (1999) and specifications of the manufacturers permit for the majority of single-ingredient and multicomponent preparations not more than 0.05% of 4-APh in paracetamol tablets (grippostad less than 0.1%, antidol and neopyrin less than 0.005%). Using the developed method it was found that the real content of 4-APh is significantly lower and amounted from 0.187×10^{-3}% in grypolek tablets to 1.566×10^{-3}% in gripex tablets. Analytical methods make it possible to detect considerably higher concentration of 4-APh; by the spectrophotometric method—more than 0.045 μg ml^{-1}, and by the HPLC with spectrophotometric detection more than 0.025 μg ml^{-1}. Thus, the above methods most frequently allow merely to find whether the content of 4-APh in the studied tablets or capsules does not exceed admissible limits. On the other hand, it is impossible to determine the real quantity of 4-APh because it is considerably below the detection limit of analytical methods. In pharmaceutical preparations, the content of 4-aminophenol is always determined in the presence of a very large excess of paracetamol and often also of dyes, preservatives, other active substances and pharmaceutical excipients. The developed HPLC method makes it possible to separate 4-APh from the matrix connected with the dosage form while the sensitivity and selectivity of the electrochemical detection enables the determination of very low concentration of 4-aminophenol in pharmaceutical preparations, higher than 4 ng ml^{-1} for tablets and capsules. Thus, HPLC-ED method is more sensitive and allows to determine the real content of 4-APh impurity in multicomponent analgesic preparations.

The statistical estimation of the results of 4-APh determination has shown that the developed HPLC-ED method for the determination of 4-APh is characterized by a good accuracy and high precision and can be applied to routine investigations of multicomponent pharmaceutical preparations in the form of tablets or capsules.

The HPLC-ED method has been validated (except for accuracy) according to the rules obligatory for analytical methods. Validation concerning the accuracy (comparison with another analytical method) was impossible because the sensitivity of the elaborated method is significantly lower than the detection limit of other analytical methods. Instead, studies on standard solutions have been performed.

4. Conclusions

An HPLC-ED procedure has been developed to provide a very sensitive, quantitative assay for low levels of the degradent 4-APh in both paracetamol drug substance and tablets or capsules formulations. This is first method that allows to determine the real content of 4-APh impurity in multicomponent analgesic preparations.

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