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Highly sensitive sequential injection determination of p-aminophenol in paracetamol formulations with 18-molybdodiphosphate heteropoly anion based on elimination of Schlieren effect

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ABSTRACT

A highly sensitive, precise and automated method using sequential injection analysis to assay quantitatively low levels of the p-aminophenol (PAP) in paracetamol formulations has been developed. A solution containing PAP and paracetamol is injected into an acetate buffer carrier stream and merged on-line with 18-molybdodiphosphate heteropoly complex reagent to form a specific blue derivative that is subsequently detected spectrophotometrically at 820 nm. The procedure has been optimized mainly with respect to measurement sensitivity. It is based on the leveling off of the refraction indices of the liquids mixed in the flow system by the careful matching of the refractive index of the reagent solution with that of the carrier and sample solutions. Amount as low as 0.5 μmol L^{-1} of PAP, which corresponds to the 0.001% of PAP in paracetamol tablets, can be reliably determined using the proposed method, which is clearly below the specification limits recommended for PAP determination in paracetamol drug and tablet formulations (50 ppm or 0.005% (w/w)). The developed method was successfully applied to the analysis of paracetamol formulations spiked with PAP and determination of PAP content in Rapidol tablets past their expiration date both by proposed SIA and reference HPLC methods.

1. Introduction

p-Aminophenol (PAP) can exist as either a synthetic intermediate in pharmaceutical preparations or as a primary hydrolytic degradation product of paracetamol. PAP is considered to be an impurity in paracetamol. It is a substance of modest toxicity, able to cause nephrotoxicity and having teratogenic effects. PAP is limited to a low level (50 ppm or 0.005% (w/w)) as a drug substance by Pharmacopoeias in Europe, the United States, Great Britain and Germany using a manual colorimetric limit test. The limit for PAP is broadened to 1000 ppm or 0.1% (w/w) for tablet product monographs, which mention the use of an automated and less sensitive HPLC method. At such a low level pharmacopoeial HPLC assay was not applicable due to matrix interference. A fast, automated assay was necessary for routine analysis. Determination of PAP is frequently used as a step in many methods based on the determination of paracetamol by its hydrolysis to PAP [1,2].

Various methods have been reported for the determination of PAP, including HPLC [3], capillary electrophoresis [4], spectrophotometry [5], fluorimetry [6] and electrochemical techniques [7,8]. Flow methods have been recognized as being potentially more sensitive and faster for the determination of PAP than HPLC analysis and other techniques [9].

FIA/SIA methods are characterized by high sensitivity, which under certain conditions can be significantly higher than that achieved with the relevant batch methods. In order to achieve low limits of detection, special attention should be paid to those factors that determine the signal-to-noise ratio. One such important factor is the Schlieren effect, which limits the sensitivity and affects the signal-to-noise ratio while impairing the reproducibility of spectrophotometric measurements [10].

The Schlieren effect is the result of light inflections caused by the formation of optical artifacts, such as when a mirror or a lens is within the flowing reaction area. The perfect mixing of a sample with reagents and carrier solvents usually cannot be achieved in flow analysis. Gradients of concentrations or sudden changes in local concentrations – that is, a difference in the refraction index along the monitored zones – lead to deflections of light that alter the intensity of the transmitted beam.

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Depending on the mixing conditions [10], the Schlieren effect can consist of two primary components. The first one is associated with the formation of stable liquid lenses – cylindrical layers having different refractive indices – under a laminar flow regime. The lenses increase or decrease the measured signal by focusing light onto or from the detector. The sequence of a positive peak followed by a negative peak, sometimes interchanged in places, is superimposed on the basic recorded output signal, thus distorting it. This component can be reproduced quite easily. The other component appears under poor mixing conditions and leads to the occurrence of a variety of transient mirrors within the flowing sample, thus leading to a noisy recorded signal and a decrease in reproducibility.

Various strategies have been proposed for the elimination of the Schlieren effect, including dual-wavelength treatment of the signal [10], matching the refraction indices of the carrier and sample solutions [11], reversed flow [12], a nested loop [13] and the introduction of large sample volumes [14].

When the dual-wavelength method is employed, the intensities of two selected monochromatic beams are measured simultaneously using separate detectors, and real-time subtraction of the wavelength-independent noise is obtained. The transmitted light is measured at two different wavelengths: one at which the product absorbs light and another outside the product's absorbance spectrum, where only the Schlieren effect is observed. Nevertheless, this methodology requires the generally complex handling of additional data and measurements at another wavelength, which together have the effect of complicating the analysis. The Schlieren effect is wavelength sensitive and noisy, thus leading to both systematic and random errors. To compensate for the Schlieren effect when using this methodology, a diode-array or CCD spectrophotometer is required, and while this method is preferable to analyzing samples having a high concentration of the analyte or other substances, the matching of the refractive index of the carrier with that of the sample and/or reagent solutions is potentially beneficial when highly concentrated carrier or reagent solutions have to be used.

This paper presents a highly sensitive, precise and automated method using Sequential Injection (SI) analysis to assay quantitatively low levels of the p-aminophenol in paracetamol formulations as degradation product. A solution containing PAP and paracetamol is injected into a buffer carrier stream and merged on-line with 18-molybdodiphosphate heteropoly complex (18-MPC) reagent to form a specific blue derivative which is subsequently detected spectrophotometrically at 820 nm. The procedure has been optimized mainly with respect to measurement sensitivity. A new strategy based on the careful matching of the refractive index of the reagent solution with that of the carrier and sample solutions has been proposed to reduce the Schlieren effect that occurs in the flow system used due to high concentrations of acetate buffer and 18-MPC reagent below the required level.

2. Materials and methods

2.1. Reagents

All reagents used were of analytical-reagent grade and distilled water was used throughout. The p-aminophenol was obtained from Sigma (St Louis, MO, USA). The PAP stock solution was prepared daily by dissolving the appropriate amount of the drug in 0.05 mol L^{-1} HCl solution to reach a final concentration of 0.01 mol L^{-1}. This solution was then stored in a refrigerator. Before being used it was diluted to the desired concentration by adding 20 mL of acetate buffer and adjusting the volume to 100 mL with water. The concentrated acetate buffer solution of pH 5.0 ± 0.2 was prepared by dissolving 10.1 g of sodium acetate in 50 mL of water, mixing in a 4.0 mL of glacial acetic acid and adjusting the resulting mixture to a volume of 100 mL. The carrier solution was prepared by using water to dilute 20 mL of concentrated buffer solution to the mark in a 100 mL volumetric flask.

The ammonium salt of the α-isomer of 18-molybdo-2-phosphate heteropolyanion $(NH_4)_6P_2Mo_{18}O_{62} \times 14H_2O$ was synthesized as described in the literature [15]. The 0.01 mol L^{-1} solution of 18-MPC was prepared by dissolving 0.7855 g of the synthesized salt and diluting it to 25 mL with distilled water. If any small insoluble residue appeared, the solution was filtered. The 1 mmol L^{-1} solution of 18-MPC was prepared in a 25 mL volumetric flask by mixing 2.5 mL of the 0.01 mol L^{-1} solution of 18-MPC with 3.0 mL of concentrated buffer solution and diluting to the mark with distilled water. This solution is unstable and should be used within 2 h of preparation.

2.2. Sequential injection system

A commercial FIAlab® 3500 system (FIAlab® Instruments, USA) with a syringe pump (syringe reservoir 5 mL) and an 6-port selection Cheminert valve (Valco Instrument Co., USA) was used. A tungsten light source and a USB 2000 UV–VIS fiber optic CCD detector (OceanOptics, USA) were connected to the flow system via 600 μm i.d. optical fibers having SMA connectors (FIAlab® Inc., Bellevue, USA). The entire SIA system was controlled using the latest version of the FIAlab program for Windows. Flow lines were made of 0.75 mm i.d. PTFE tubing. 10, 20 and 50 mm optical Z-flow through cells were used.

2.3. HPLC apparatus

The comparative HPLC system, made up of a binary pump LCP 4100 (Ecom, Prague), a Waters 717 plus auto sampler, a Waters 486 Tunable UV detector (Waters, Milford, MA) and a PC for data processing, was controlled by the chromatographic software CSW v.1.7 for Windows (Data Apex s.r.o., Prague). Sample compounds separation was performed on an Onyx Monolithic C18, with a 50 mm × 4.6 mm column (Merck, Germany). The optimal mobile phase for the separation of p-aminophenol and paracetamol was a mixture of acetonitrile:water in a 10:90 (v/v) ratio, with the pH adjusted to 2.8 through the use of orthophosphoric acid (8.5%). Helium was used to degas the mobile phase prior to application.

The final optimized conditions selected were as follows: injection volume of 10 μL for the PAP sample; the isocratic mobile phase was pumped at a flow rate of 1 mL min^{-1} at ambient temperature; and the detection wavelength was 210 nm. A 0.05 mg mL^{-1} solution of caffeine was used as the internal standard.

2.4. General SIA procedure

The configuration of the SIA manifold employed for the determination of PAP is shown in Fig. 1. The analytical cycle began by filling the piston pump syringe with 1000 μL of the carrier solution (an acetate buffer solution), which was drawn into the syringe at a flow rate of 50 μL s^{-1}. This was followed by 75 μL of reagent (1 mmol L^{-1} M solution of 18-MPC in acetate buffer) and 160 μL sample or PAP standard aspirated sequentially into the holding coil at 30 μL s^{-1} through separate ports (ports no. 2 and no. 3, respectively) of the multi-position valve. The entire volume was then propelled at 30 μL s^{-1} through the Z-flow cell using port no. 6. A spectrometer reference scan was made, and absorbance scanning began immediately.

2.5. Determination of PAP in paracetamol formulations

Five paracetamol tablets were accurately weighed and crushed into a powder. An amount equivalent to one tablet (500 mg) was