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Determination of paracetamol and its main impurity 4-aminophenol in analgesic preparations by micellar electrokinetic chromatography

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ABSTRACT

A micellar electrokinetic chromatographic (MEKC) method was developed for the quality control of paracetamol containing pharmaceutical preparations. The influence of several factors (surfactant concentration, buffer concentration, pH and applied voltage) was studied during development and optimisation of the method. Phosphate buffer (pH 9.0) containing sodium dodecyl sulphate (75 mM) was found as the ideal running buffer for the separation; the applied voltage was 25 kV and the analysis time was 10 min. The limit of quantitation for 4-aminophenol was 6 μg ml^{-1}; the linearity of the method was studied in the concentration ranges 20–260 μg ml^{-1} for paracetamol and 20–150 μg ml^{-1} for 4-aminophenol. The method was successfully applied for the quality control of paracetamol containing products.

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1. Introduction

Paracetamol is a widely used analgesic drug formulated in a variety of dosage forms that are available as OTC products. It is used in the treatment of pain and fever but it has no anti-inflammatory activity [1]. 4-Aminophenol (*p*-aminophenol; AP) is a degradation product of paracetamol or it may be originated from the synthesis; it is reported to have significant nephrotoxic and teratogenic effects, therefore its amount should be strictly controlled. It is limited to a low level of 0.005% in the drug substance by the European Pharmacopoeia (Ph. Eur.) [2]. The limits for AP may vary in different products depending on the dosage form and formulation; the monograph of paracetamol tablets in BP allows 0.1% [3]. Limits for other impurities of paracetamol are usually not prescribed for products as those do not originate from degradation and their amount is limited in the substance by Pharmacopoeias.

For the quality control of paracetamol containing products, a variety of methods are described in the literature. Usually two different methods are used for the assay and testing for AP. The tablet product monograph of the British Pharmacopoeia contains a spectrophotometric assay method for paracetamol and an HPLC method for the determination of AP [3] while the monograph of USP 30 contains an HPLC assay method but no impurity testing method [4]. Several methods (RP-HPLC, microemulsion liquid chromatography, capillary electrophoresis, UV spectrophotometry) have been published [5–18] on the assay of paracetamol in preparations, some of these works deal with the simultaneous determination of AP in the products [9–18]. A remarkable study has been also published about the determination of AP by quantitative ^{1}H NMR spectroscopy [19]. A useful overview is presented in [20] about the different possibilities for the determination of paracetamol content in pharmaceutical products and biological samples.

HPLC methods [10–15] are suitable for the intended purpose, although some have their drawbacks: special columns, for example a carbon-based column [11] or polyethylene glycol column [14] or previous derivative formation is needed [12], which increases the costs and the length of the analytical procedure. Amperometric detection is applied in [13], which is not generally available in analytical laboratories. Methods [10] and [15] use C$_{18}$ stationary phase with UV detection, the drawback of this type of columns is that the chromatographic performance of different brands may differ considerably. The aim of our work was to study the applicability of capillary electrophoresis for this purpose and to develop an alternative method which is fast and cost-effective compared to the existing methods.

Although some CE methods have already been published for the analysis of paracetamol containing products [16–18], they were not easily applicable in practice. Some of them were developed for a special kind of equipment (CE with amperometric detection)

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[16–17] while using the method of Pérez-Ruiz et al. [18], AP cannot be quantified at the concentrations it can be present in real samples due to the overlapping of the peaks (0.5% compared to paracetamol is an extremely high amount). The CE methods [16–17] propose the usage of a phosphate or borate–phosphate buffer adjusted to 6.5 and 7.2, respectively; under these circumstances paracetamol is not charged due to its pKa of 9.55 [21], therefore its assay in pharmaceutical products may have difficulties because of the presence of other neutral substances like pharmaceutical excipients.

Several research groups were interested in the CE determination of 4-aminophenol in the presence of other phenol derivatives as well [22–24], Gotti et al. developed a method to quantify 4-aminophenol as an impurity of mesalazine [25].

The insufficiency of method [18] was caused by the fact that under alkaline conditions the electrophoretic mobility of paracetamol and AP is only slightly different (at neutral and acidic pH the paracetamol molecule is uncharged and its determination can be a problem due to other neutral substances, as mentioned above). As CZE does not allow an appropriate separation and peaks are overlapped at higher concentrations, we tried to add sodium dodecyl sulphate to the running buffer, to get a better separation by micellar electrokinetic chromatography (MEKC). MEKC seemed to be more appropriate for this separation than CZE.

2. Experimental

2.1. Apparatus

Analysis was performed with a Hewlett-Packard Model G1600AX [3D] CE system equipped with diode-array detector and was controlled by ChemStation software. The separation was performed in an uncoated fused silica capillary with a total length of 64.5 cm and effective length of 56 cm (50 μm i.d.) with a bubble cell (optical path length 150 μm). The cartridge in which the capillary was housed was thermostated at 20 °C. Samples were injected hydrodynamically using a pressure of 50 mbar for 6 s. Separations were carried out using normal polarity at an applied voltage of 25 kV. Detection was carried out at 240 nm.

Background electrolyte was prepared by dissolving sodium dodecyl sulphate (75 mM) and sodium dihydrogen phosphate (50 mM) adjusted to pH 9.0 using 50 mM of trisodium phosphate.

Capillary was conditioned daily prior to analysis by 1 M NaOH for 15 min, by water for 5 min and then with the running buffer for 15 min. Between runs the capillary was equilibrated with the running buffer for 4 min.

2.2. Chemicals

Acetonitrile (HPLC grade) was purchased from Carlo Erba. Sodium dihydrogen phosphate, trisodium phosphate and sodium dodecyl sulphate were supplied by different manufacturers (Acidum-2, Reanal, Chemolab BO) in analytical grade. Water was produced by a Millipore Elix 3 water cleaning system. 4-Aminophenol was purchased from Merck while paracetamol was a secondary reference substance from the Sanofi-Wintrop Company.

2.3. Preparation of samples

Ten tablets were weighed and finely powdered; an amount equivalent to 1.0 g of paracetamol was suspended in 50 ml of a mixture of acetonitrile and water (15:85, v/v) by ultrasonication for 30 min. After the sonication, the sample was filtered. For the assay, the filtered sample was 100-fold diluted with the solvent mixture; the undiluted sample was used for the determination of impurities.

3. Results and discussion

3.1. Effect of sodium dodecyl sulphate concentration to the separation

In the MEKC method development, the optimization of detergent concentration is of major importance. Increasing the concentration, the higher amount of micelles results in a more effective separation but it also increases the current in the capillary. The effect of SDS concentrations of 25–150 mM was investigated. The concentration of 75 mM was found to be optimal: lower concentrations decreased the separation while using higher concentrations, the separation voltage had to be reduced (but a lower voltage results in longer migration times).

3.2. Effect of buffer concentration and pH

The increase of pH in the range of 7.0–9.0 results in increasing separation while further increase of the pH has no significant consequence on separation (but it could be realized that strongly alkaline condition results in the decomposition of AP—it is a phenolic compound, therefore it is sensitive to oxygen, especially under alkaline conditions, see the degradation pathway in [27]). The increase of buffer concentration from 20 to 50 mM improves peak symmetry but the further increase generates a high current, therefore pH 9.0 and the phosphate buffer concentration of 50 mM was found to be optimal.

3.3. Influence of applied voltage

Increasing the applied voltage leads to shorter run times and higher efficiencies. However, higher voltages produce high current and increase Joule heating, which is unfavourable for the stability of AP during the analysis. The voltage range of 15–30 kV was tested under the separation conditions selected above. The voltage of 25 kV was found optimal (current 80 μA).

3.4. Method validation

In accordance with the International Conference of Harmonization (ICH) [28], this method was validated by determination of the specificity, linearity, precision, accuracy, limit of quantitation and robustness.

3.4.1. Specificity

The specificity of the method was demonstrated by recovery experiments with the products spiked with AP, to study the possible interference of excipients. No electrophoretic interference was found. As migration times were reproducible, the method allowed the discrimination of paracetamol and AP. Fig. 1 represents the electropherogram of a product spiked with 0.3% 4-aminophenol while Fig. 2 shows the electropherogram of the diluted sample. It can be seen that there is no interference from excipients, which allows the assay of paracetamol with this method.

3.4.2. Linearity

The linearity of the method was examined in the range 20–150 μg ml^{-1} for AP and 20–260 μg ml^{-1} for paracetamol. External calibration was used. The calibration curves can be described using the following equations: $y = (2.2 \times 10^{-3} \pm 8.3 \times 10^{-3}) + (2.6 \times 10^{-3} \pm 5.1 \times 10^{-5})x$, $r^2 = 0.9978$ for paracetamol ($n = 8$) and $y = (8.4 \times 10^{-4} \pm 1.3 \times 10^{-3}) + (6.8 \times 10^{-4} \pm 1.4 \times 10^{-5})x$, $r^2 = 0.9987$ for AP ($n = 5$), where y is the peak area and x is the concentration in μg ml^{-1}.
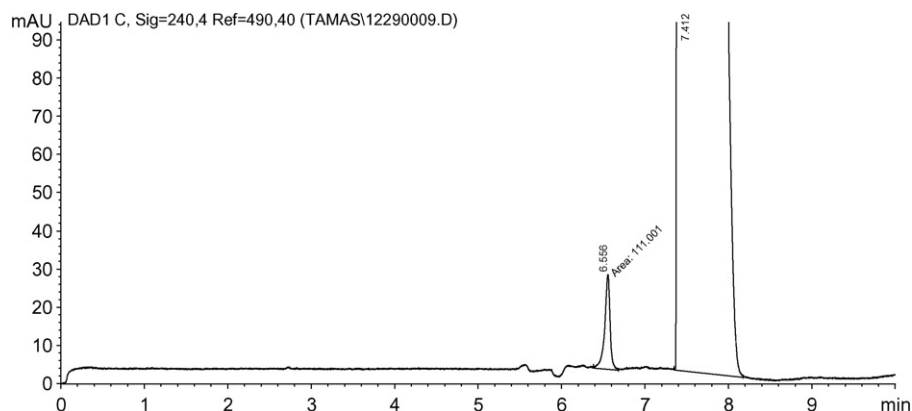
Fig. 1. Separation of paracetamol (20 mg ml^{-1}) and 4-aminophenol (60 μg ml^{-1}) in a spiked pharmaceutical product. For the experimental conditions see *Apparatus*.
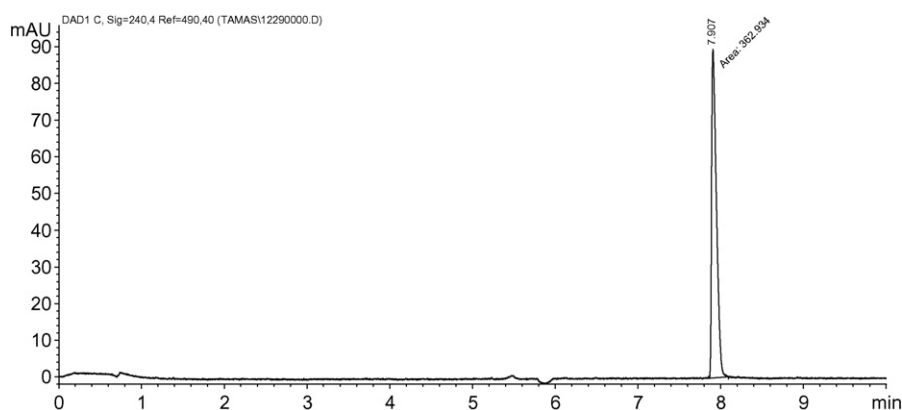

Fig. 2. Assay of a paracetamol containing product (200 μg ml^{-1} paracetamol). For the experimental conditions see *Apparatus*.

3.4.3. Precision

The system precision of the method was examined by injecting the solution containing 200 μg ml^{-1} of paracetamol six times within a day. The precision of peak area and migration time (as relative standard deviation, R.S.D., $n = 6$) was 0.32 and 0.42%, respectively.

The repeatability was examined by multiple sampling of the same homogenous sample (tablet product): six solutions containing 200 μg ml^{-1} of paracetamol were prepared and injected within a day, the R.S.D. for peak area was 1.78% for paracetamol.

3.4.4. Accuracy

The accuracy of the method for the assay was tested by comparing the results with those of the USP assay method as reference method (Table 1). The accuracy for impurity testing was justified by the analysis of products spiked with different amounts of AP. 3 different concentrations were used (60, 80 and 100 μg ml^{-1}, representing 0.3, 0.4 and 0.5% compared to 20 mg ml^{-1} paracetamol) and the recoveries were between 99.9 and 104.1%.

Table 1
Determination of the paracetamol content of pharmaceutical products (tablets) using the proposed MEKC method and the HPLC assay method of USP [4]

	Stated amount of the active compound (mg/tablet)	Amount found (mean ± S.D.)	
		MEKC method	HPLC method
1. Product	500	511.58 ± 7.5	500.16 ± 2.1
2. Product	500	493.6 ± 3.8	495.96 ± 0.8
3. Product	250	237.95 ± 0.5	236.99 ± 1.8

In the 3 tested products AP was not found in a detectable amount (LOD (S/N = 3) value can be estimated from LOQ (S/N = 10) value: 2 μg ml^{-1} for 4-aminophenol).

3.4.5. Limit of quantitation (LOQ)

LOQ (S/N = 10) was found to be 6 μg ml^{-1} for AP (R.S.D. 5.7% for LOQ). According to the ICH guideline [28], the determination of LOQ is not necessary for assays; therefore it was not determined for paracetamol. As the peaks of impurities and paracetamol are well separated, impurities can be quantified at low concentrations, using concentrated sample solutions. In a solution containing 20 mg ml^{-1} of paracetamol, the quantitation limit corresponds to 0.03% 4-aminophenol. Comparing our method to the HPLC method prescribed in BP for the determination of AP, this quantitation limit is low enough as BP prescribes 0.1% for the limit of this impurity in paracetamol tablets.

3.4.6. Robustness

The robustness of the method was demonstrated by studying the influence of the variation of method parameters on the separation. The influence of changing the SDS concentration and pH of the buffer was examined and it was found that varying these values in a ±10% range has only minor influence on the efficiency and resolution of the compounds.

4. Conclusions

MEKC enabled a powerful separation of the impurity 4-aminophenol from paracetamol within 10 min run time. Our

method has been shown to be selective (no interference was shown by excipients) and sufficiently sensitive; after optimizing the method, it was applicable to the analysis of commercially available products.

Acknowledgements

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