ELSEVIER

Journal of Chromatography A, 924 (2001) 465–470

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Selective and quantitative analysis of 4-hydroxybenzoate preservatives by microemulsion electrokinetic chromatography

P.-E. Mahuzier[a,b,*], K.D. Altria[a], B.J. Clark[b]

[a]*GlaxoWellcome Research and Development, Ware, Herts, SG12 0DP, UK*

[b]*Drug Development, Bradford School of Pharmacy, University of Bradford, Bradford, West Yorkshire BD7 1DP, UK*

Abstract

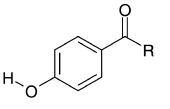
A microemulsion electrokinetic chromatography (MEEKC) method has been developed and validated for the determination of 4-hydroxybenzoates and their impurities. These materials are commonly known as parabens and are widely used as preservatives in foods, cosmetics and pharmaceuticals. The method was shown to be selective and quantitative for the methyl, ethyl, propyl and butyl esters of 4-hydroxybenzoic acid. An internal standard, 4-hydroxyacetophenone, was employed to improve injection precision and detector linearity. In addition, 4-hydroxybenzoic acid, the major degradent, could also be monitored at the 0.1% (m/m) level. The method was successfully validated for assay and detection of the impurities in 4-hydroxybenzoic acid methyl ester and 4-hydroxybenzoic acid propyl ester samples and for the determination of 4-hydroxybenzoic acid methyl ester in a liquid pharmaceutical formulation. The determination of paraben content by MEEKC in a liquid sample was consistent with HPLC analysis. This work is the first reported validated MEEKC method and shows that the methodology can be successfully implemented into routine quality control testing.

Keywords: Microemulsion electrokinetic chromatography; Validation; Parabens; Hydroxybenzoic acid

1. Introduction

Food products, cosmetics and pharmaceuticals use 4-hydroxybenzoates (parabens) as antimicrobial preservatives. These compounds are esters of 4-hydroxybenzoic acid (Fig. 1). The methyl and propyl esters are the most frequently employed parabens. These materials can contain levels of ethyl, butyl parabens and 4-hydroxybenzoic acid as impurities. The European Pharmacopoeia [1] test for 4-hydroxybenzoate preservatives is a non-selective assay by titration following sample saponification, and a TLC impurity method, which is not quantitative. Chromatographic methods such as high-performance liquid chromatography (HPLC) have also been reported to assay methyl paraben and propyl parabens [2].

There has been limited use of capillary electrophoresis (CE) in these assays. Recently there has been increased investigation into the use of microemulsion capillary electrophoresis (MEEKC) [3,4] in CE separations. MEEKC methods have been



R: -OH (4-hydroxybenzoic acid), -O-CH_3 (methyl), -O-C_2H_5 (ethyl), -O-C_3H_7 (propyl), -O-C_4H_{11} (butyl)

Fig. 1. Structure of parabens.

*Corresponding author. Present address: GlaxoWellcome Research and Development, Ware, Herts, SG12 0DP, UK.

0021-9673/01/$ – see front matter
PII: S0021-9673(01)00717-8

shown [5,6] to be appropriate for a wide range of sample types when using a microemulsion containing octane–butan-1-ol–SDS–borate (pH 9.5).

For the assay of the 4-hydroxybenzoates and their main impurities a MEEKC method was developed, with the purpose of developing a suitable method which could be used for routine quantitative assays in a pharmaceutical industrial quality control laboratory.

2. Experimental

The assays were performed with an HP^{3D}CE Agilent G1600AX (Agilent Technologies, Bracknell, UK) instrument interfaced with an Atlas 99 Labsystems data collection system (Altrincham, UK) which provided integration and data handling. Inorganic chemicals were obtained from BDH (Poole, UK) and organic chemicals from Sigma–Aldrich (Poole, UK). Water was obtained from a Millipore Milli-Q system (Watford, UK). Capillaries were purchased from Agilent Technologies and were 32 cm×50 μm I.D. and connected to a ×3 bubble cell fused-silica capillary, with the detection window at 21 cm. New capillaries were pre-conditioned prior to use by rinsing for 20 min with 0.1 *M* sodium hydroxide.

The standard microemulsion operating solution was prepared by weighing octane (0.81%, m/m), butan-1-ol (6.61%, m/m), sodium dodecylsulphate (SDS) (3.31%, m/m) and sodium tetraborate aqueous buffer (10 m*M*) (89.27%, m/m) (pH 9.5) into a 100-ml flask and sonicating the mixture for 30 min to aid dissolution.

A low pH microemulsion solution was prepared from the standard microemulsion solution above using sodium phosphate (50 m*M*, pH 2.1) rather than the sodium borate buffer. Sodium phosphate buffer (50 m*M*, pH 2.1) was prepared by dissolving 4.9 g of phosphoric acid in ~900 ml of pure water, and titrating to pH 2.1 with 1 *M* sodium hydroxide. This solution was then made up to 1000 ml with pure water.

To increase solubility of the parabens in the microemulsion, methanol was added in the sample preparation step. In these experiments the maximum concentration of methanol which could be added into the microemulsion [4] was 8% (v/v). Above this level segregation of the emulsion occurred. The sample diluent was therefore composed with microemulsion and methanol (92:8%, v/v) which was sonicated for 15 min.

For impurities testing, a test mixture of four parabens: butyl paraben, propyl paraben, ethyl paraben, methyl paraben and 4-hydroxyacetophenone was used in the method development. Each component was dissolved in sample diluent to give a 5 mg ml^{-1} solution. The main impurity, 4-hydoxybenzoic acid, was also added to the mixture at 5 mg ml^{-1}. The solution was then sonicated for 15 min.

Sample concentrations of 5 mg ml^{-1} in diluent were used for impurity analysis, while 1 mg ml^{-1} concentrations of parabens in diluent containing internal standard were used for assay purposes. An internal standard solution of 4-hydroxyacetophenone (1 mg ml^{-1}) was prepared in sample diluent when performing assays. 4-Hydroxyacetophenone was suitable as it did not react with either the sample or the microemulsion and has a good UV response (maximum absorbance at 200 nm).

Both sample and calibration solutions of methyl paraben and propyl paraben, for assay and linearity, were prepared in sample diluent containing internal standard and these solutions were sonicated for 15 min.

A commercial pharmaceutical preparation of syrup containing digoxin was used to test the suitability of the assay for industrial samples. A solution was prepared by taking 5 ml of syrup containing digoxin into 10 ml of internal standard solution, to give a final concentration of 0.5 mg ml^{-1} and the solution was sonicated for 15 min.

3. Results and discussion

3.1. Method development

With the borate microemulsion solutions at high pH [5,6], migration order was methyl, ethyl, propyl, and then butyl paraben. The migration order followed the pattern of the more water-soluble paraben eluting first. However, the pH of the borate buffer (pH 9.2) and the pK_a of 4-hydroxybenzoic acid (pK_a~8.4) were incompatible and the 4-hydroxy-

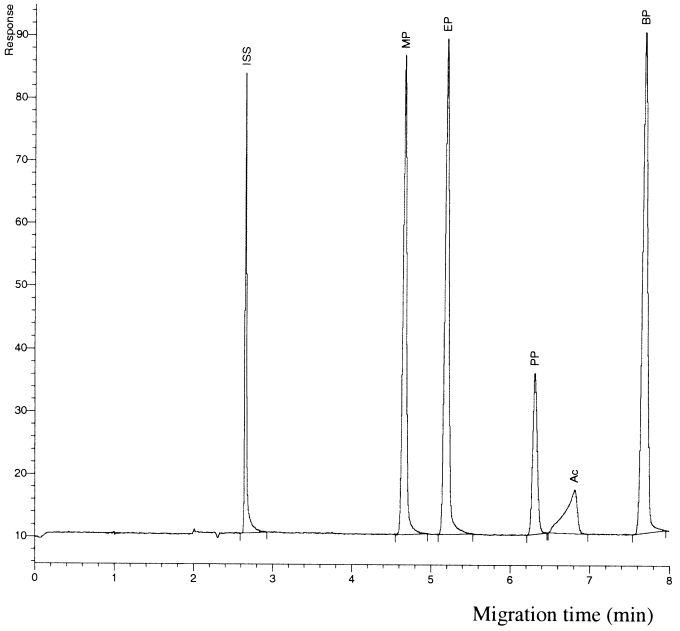


Fig. 2. Separation of a test mixture in high pH microemulsion (pH 9.5) by MEEKC. The sample was injected for 3 s at 20 mbar. Separation conditions: 0.81% (m/m) octane, 6.61% (m/m) butan-1-ol, 3.31% (m/m) sodium dodecylsulphate and 89.27% (m/m) 10 m*M* sodium tetraborate buffer, 11 kV, 33 cm×50 μm I.D. capillary (detection window at 25 cm), 40°C, 200 nm. Sodium hydroxide was rinsed for 1 min and then the running buffer was rinsed for 1 min through the capillary between each run. Elution order: internal standard (ISS), methyl paraben (MP), ethyl paraben (EP), propyl paraben (PP), 4-hydroxybenzoic acid (Ac), butyl paraben (BP).

benzoic acid gave a poor peak (Fig. 2) due to the co-existence of 4-hydroxybenzoic acid and 4-hydroxybenzoate species. Previous work [7] has shown the possible advantages of operating MEEKC methods at low pH. The pH was therefore lowered to pH 2.1, which ensured that both the parabens and 4-hydroxybenzoic acid were neutral and gave improved peak shape. Under these conditions the migration order was changed and the more insoluble butyl paraben was eluted first, followed by propyl, ethyl, methyl paraben and finally the main impurity with the components had acceptable peak shape (Fig. 3). Work with this low pH microemulsion buffer was therefore continued and the method was validated.

3.2. Method validation

3.2.1. Injection precision

A total of ten injections of the test mixture (5 mg ml^{-1}) were performed to demonstrate the injection precision for each paraben. The mean peak area ratios (PAR) against internal standard were RSD $<1.5\%$ with the individual values given in Table 1. Good selectivity was given for the different parabens and an acceptable resolution maintained with relative migration time (RMT) precisions RSD $<0.2\%$.

3.2.2. Concentration linearity for the parabens

The linearity of the parabens at 1 mg ml^{-1} was performed for 50–150% (m/m) of the nominal concentration (0.5–1.5 mg ml^{-1}). The correlation coefficients obtained were satisfactory (0.9998 for methyl paraben and 0.9999 for propyl paraben). Impurities linearity was also performed over the range 0.1–1% (m/m) of the nominal concentration range. The correlation coefficients obtained were also satisfactory (0.995 for impurities level). The use of an internal standard (4-hydroxyacetophenone) improves both injection precision and detector linearity. The linearity improvement is due to a reduction in the scatter of the point along the line. Linearity experiments were found to be successful with correlation coefficients close to the limits stipulated in the method development concentration.

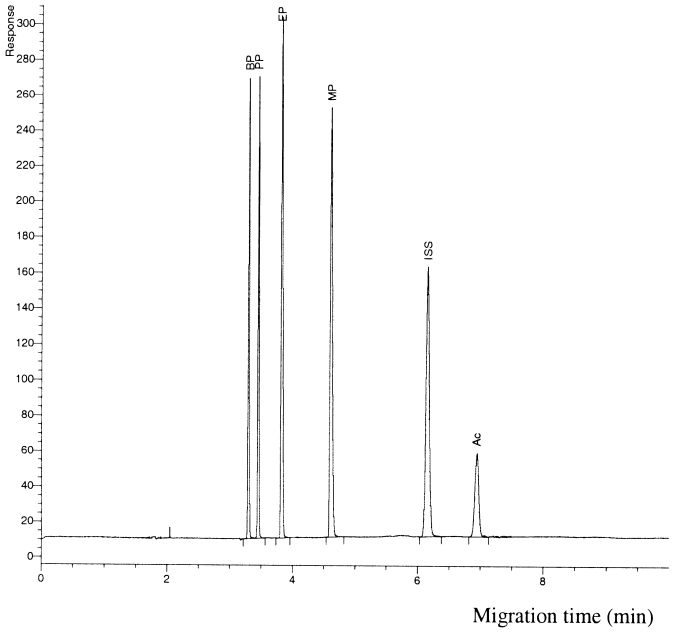


Fig. 3. Separation of a test mixture in low pH microemulsion (pH 2.1) by MEEKC. The sample was injected for 3 s at 20 mbar. Separation conditions: 0.81% (m/m) octane, 6.61% (m/m) butan-1-ol, 3.31% (m/m) sodium dodecylsulphate and 89.27% (m/m) 50 m*M* phosphate buffer, pH 2.1, −11 kV, 33 cm×50 μm I.D. capillary (detection window at 25 cm), 40°C, 200 nm. The running buffer was rinsed through the capillary for 2 min between each run. Elution order: butyl paraben (BP), propyl paraben (PP), ethyl paraben (EP), methyl paraben (MP), internal standard (ISS) and 4-hydroxybenzoic acid (Ac).

3.2.3. Measurement of ruggedness

The method was repeated on several occasions using different capillaries, standard, and sample solutions. Different microemulsion preparations were used and the method was repeated on two different instrument designs.

3.2.4. Recovery

A sample of propyl paraben (5 mg ml^{-1}) was spiked with 5 μg ml^{-1} of each of methyl, ethyl, and butyl paraben equivalent to 0.1% (m/m) to demonstrate component recovery. Fig. 4 shows the separation of propyl paraben and its impurities. Recovery was successful as methyl, ethyl, butyl, and 4-hydroxybenzoic acid were detected with their area being 0.1% of the area of the propyl paraben peak.

Table 1
Injection precision

	RSD (%)	
	Relative migration time	Peak area ratio
Butyl paraben	0.12	0.85
Propyl paraben	0.13	1.05
Ethyl paraben	0.17	1.00
Methyl paraben	0.12	0.46

3.2.5. Limit of detection

The limit of detection (LOD) in this method validation was set at 0.1% of the main peak. LOD is defined as the smallest peak detected with a signal height three times that of the baseline. Fig. 4 shows detection of 0.1% impurities in a propyl paraben sample. The sensitivity check solution containing the parabens and the 4-hydroxybenzoic acid was prepared by diluting a 5 mg ml^{-1} sample solution to obtain an area equivalent to 0.1% of the normal concentration test-mixture peak area.

3.2.6. Determination of the limit of quantitation

Limit of quantitation (LOQ) refers to the lowest level of material which can be determined with an

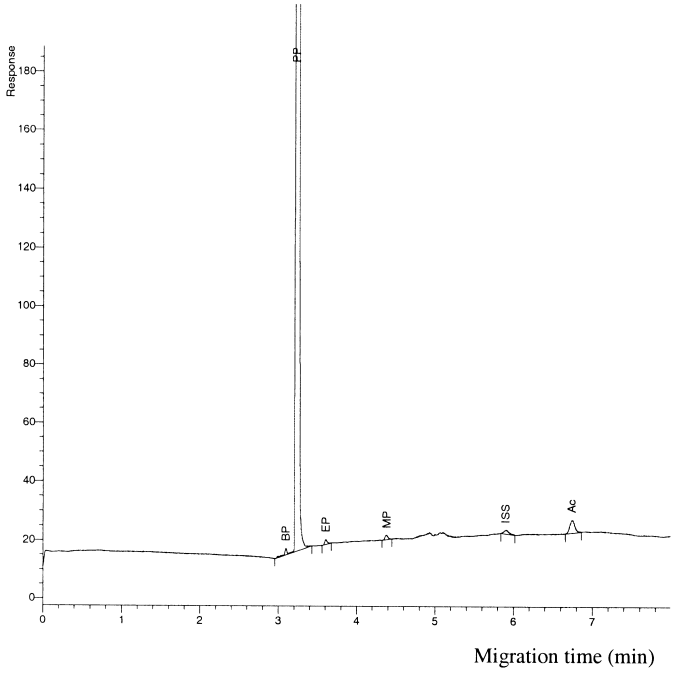


Fig. 4. Demonstration of the separation of propyl paraben with 0.1% of butyl, ethyl, propyl, paraben and the main impurity. Separation conditions as Fig. 3.

acceptable degree of confidence. LOQ value is often calculated as ten times the signal height to the baseline. A test mixture (5 mg ml^{-1}) was diluted to 0.2% (m/m) and injected ten times. To fit the acceptance criteria the peak area ratio must be below 20% RSD. The experiment gave a maximum value of 16% RSD, therefore the limit of quantitation was satisfactory.

3.2.7. Assay of paraben batches

Industrial samples of bulk methyl and propyl paraben were assayed against reference standard solutions of parabens. Methyl, propyl paraben solutions were prepared at a concentration of 1 mg ml^{-1}. Results obtained (Table 2) demonstrate that the MEEKC method was suitable for determination of paraben concentrations (the specified limits were 90–110% of the nominal concentration). The assay was also found to be successful, the samples analysed were within tolerances limits, and no impurities were detected in the batches above the LOD of 0.1% (m/m).

3.3. Method application

3.3.1. Assay of methyl paraben content in a liquid syrup containing digoxin

Digoxin is a cardiac glycoside used for treating low output congestive heart failure and cardiac arrhythmias [8]. Samples of syrup containing digoxin were assayed (Fig. 5) using an analytical reference standard of paraben. Methyl paraben calibration solutions were prepared at a concentration of 1 mg ml^{-1}. Prior to the assay, injection precision was assessed with a standard solution; the RSD of peak

Table 2
Assay results for industrial samples of paraben

	% (m/m)
Methyl paraben	
Sample 1	99.3
Sample 2	100.2
Propyl paraben	
Sample 1	100.8
Sample 2	102.3

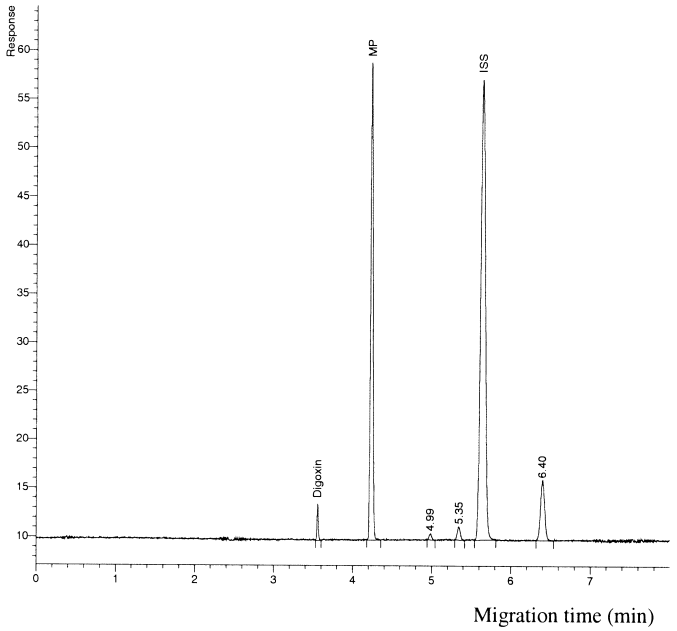


Fig. 5. Separation of the components present in the liquid pharmaceutical sample by MEEKC. The sample was diluted with microemulsion containing the internal standard and injected for 3 s at 20 mbar. Separation conditions: 0.81% (m/m) octane, 6.61% (m/m) butan-1-ol, 3.31% (m/m) sodium dodecylsulphate and 89.27% (m/m) 50 m*M* phosphate buffer, pH 2.1, −11 kV, 33 cm×50 μm I.D. capillary (detection window at 25 cm), 40°C, wavelength 200 nm. The running buffer was rinsed through the capillary for 2 min between each run. The elution order was digoxin, methyl paraben (MP), and internal standard (ISS).

area ratio was 0.9%. The results demonstrated that the MEEKC method was suitable for determination of paraben concentrations. The assay was found to be successful and the data obtained were comparable to a validated HPLC method (CE, 0.738 mg ml^{-1}; HPLC, 0.736 mg ml^{-1}).

4. Conclusion

A CE method has been developed and validated for methyl, ethyl, propyl and butyl parabens. The method employs 4-hydroxyacetophenone as an internal standard. Successful validation included linearity, injection precision, sensitivity and assay of methyl paraben and propyl paraben. The method is suitable for monitoring other parabens as they can be present at low levels in a batch of pure methyl or propyl paraben or in a liquid pharmaceutical formulation.

References

[1] European Pharmacopeia Supplement, 3rd ed., Council of Europe, Strasbourg, 2001, p. 523, 794, 1125, 1336.
[2] M.A. Moreno, D. Castro, P. Frutos, M.P. Ballesteros, J.L. Lastres, Chromatographia 52 (2000) 589.
[3] K.D. Altria, J. Chromatogr. A 892 (2000) 171.
[4] K.D. Altria, B.J. Clark, P.-E. Mahuzier, Chromatographia 52 (2000) 758.
[5] K.D. Altria, J. Chromatogr. A 844 (1999) 371.
[6] K.D. Altria, Chromatographia 49 (1999) 457.
[7] S. Pedersen-Bjergaard, Ø. Næss, S. Moestue, J. Chromatogr. A 876 (2000) 201.
[8] J. Reynolds, Martindale, 30th ed., Pharmaceutical Press, London, 1993, p. 1664.