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Isocratic liquid chromatographic determination of three paraben preservatives in hygiene wipes using a reversed phase core-shell narrow-bore column

Short Communication

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Abstract: The first HPLC method for the separation of three paraben preservatives (methyl-, ethyl- and propyl parabens) using a core-shell analytical column is reported in this study. The separation was completed in less than 8 min at a low flow rate of 0.4 mL min^{-1} and an isocratic mobile phase containing 20% acetonitrile as organic modifier. The backpressure was < 200 bar in all cases, enabling the usage of conventional HPLC equipment. The proposed analytical procedure was validated for linearity (0.5 – 20 mg L^{-1}), limits of detection (15 – 43 μg L^{-1}) and quantification (50 – 142 μg L^{-1}), selectivity, within day (1.3 – 1.5%) and day-to-day (3.4 – 4.6%) precision and accuracy. The proposed method has been applied to the determination of the selected paraben preservatives in commercially available hygiene wipes. The mean percent recoveries were found to be in the range of 98.0 - 98.4%.

Keywords: *High-performance liquid chromatography • Core-shell (or fused core) column • Parabens • Hygiene wipes*

1. Introduction

Fast liquid chromatography (LC) is one of the most "hot" trends in modern separation science. This trend is dictated by the demand of information gathering at the shortest possible time and by the continuously growing amount of samples. From a practical point of view, fast LC can be mainly applied through Ultra High Pressure LC (UHPLC) using sub 2-μm particulate columns, high temperature LC and low pressure monolithic materials that allow elevated flow rates. The special requirements in terms of instrumentation and advantages / disadvantages of these approaches are well known and adequately discussed in the literature [1].

Recently, the new trend in fast LC technology is the development of analytical columns consisting of core-shell particles. Structurally, these materials consist of a non-porous core, surrounded by a porous solid shell and their chromatographic properties are more or less governed by the diameter of the core and by the thickness of the external shell [2,3]. Although the idea of using core-shell particles for liquid phase separations is far from being considered as new, only very recently many manufacturers have launched related products. The main advantages of core-shell particles include: (i) fast mass transfer kinetics, (ii) high resolution separations comparable or even better than sub 2-μm columns and (iii) moderate operating pressures that expand the capabilities of conventional HPLC instrumentation.

Paraben preservatives are – from a chemical point of view – esters of p-hydroxybenzoic acid which are widely used in food, pharmaceutical and cosmetic industries [4]. Potential hazards from the use of parabens in widely consumed products are under investigation, but

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the results are not yet conclusive. For example, in a recent study the authors claimed existence of traces of parabens in breast tumors [5]. On the other hand, other reports support the safety of this group of preservatives proving very low estrogen-like activity [6]. Nevertheless, international authorities have set limits for the presence of parabens; for example, in cosmetic products the maximum allowed concentration for total parabens is 0.8% and for single parabens is 0.4% w/w [7].

On-going research and debate on parabens effects and toxicity has led to the development of various methods for their determination in various matrices such as food, biological material, pharmaceuticals, cosmetics, even in environmental samples. The recently reported analytical procedures cover a wide range of separation techniques such as HPLC [8,9], UHPLC [10], GC [11], MEKC [12] just to name some. Each approach has pros and cons depending on the selected technique and detection. For example, conventional HPLC using 5-μm particulate columns under isocratic elution requires at least 15-20 min for the elution of propyl paraben [8] or suffers from high mobile phase consumption using monolithic columns [9]; UHPLC offers rapidity, but sophisticated instrumentation is necessary to take full advantage of the capabilities of sub 2-μm particles [10]; GC-MS is fast and effective in terms of separation, but requires long derivatization time at elevated temperatures [11]; MEKC on the other hand, can be certainly considered as an environmental friendly technique offering minimal consumption of reagents, but it suffers from low sensitivity and LOQs at the ppm level [12].

To the best of our knowledge there is no previous report on the separation and determination of parabens by HPLC employing a core-shell particulate column. The scope of this study was therefore to demonstrate the usefulness of this new type of analytical columns to the analysis of three paraben preservatives, namely methyl- (MP), ethyl- (EP) and propyl- (PP) parabens. The developed and validated analytical procedure has been applied to the determination of the selected preservatives in commercially available hygiene wipes.

2. Experimental procedure

2.1. Reagents and solutions

Ultra-pure water was produced by a Milli-Q (Millipore) system. HPLC grade methanol and acetonitrile were used during all experiments (Merck, Darmstadt, Germany).

Methylparaben (MP), ethylparaben (EP), and propylparaben (PP) reference compounds (purity > 99%) were donated by Cosmopharm Ltd (Greece). Phenoxyethanol was also provided by Cosmopharm Ltd (purity > 99%).

Standard stock solutions of the analytes were prepared in MeOH at the 500 mg L^{-1} level and kept refrigerated. The stock solutions were found to be stable for at least two weeks. Working mixtures were prepared freshly by dilution in aqueous solutions of methanol (φ = 50%).

2.2. Instrumentation and materials

The HPLC setup comprised the following parts: a AS3000 autosampler including a column oven (Thermo Scientific); a LC-9A binary pump (Shimadzu); a SPD-10A UV-Vis detector (Shimadzu) and an Elite™ vacuum degasser (Alltech). Data acquisition was carried out *via* the Clarity® software (DataApex, Czech Republic).

An Accucore C18 core-shell narrow-bore analytical LC column (50×2.1 mm i.d., Thermo Scientific) was used throughout the study.

2.3. HPLC procedure

Three microliters of the samples or standards were injected in the analytical column. The three paraben preservatives were separated at a flow rate of 0.4 mL min^{-1} and they were detected at 254 nm. A mixture of acetonitrile / 0.1% acetic acid (20 : 80 v/v) was used as isocratic mobile phase. The column was thermostated at 40°C throughout this study. Under the above-mentioned conditions, the analysis cycle was completed in 8 min, while each sample or standard was injected in triplicate.

2.4. Sample preparation

Hygiene wipes samples were purchased from the local market and they were processed directly after unpacking. A first treatment step involved the drying of the samples for 24 h at 50°C until constant weighted. Subsequently, accurately weighed pieces of the wipes (ca. 1 g) were treated with 10 mL methanol, followed by ultrasonication for 15 min and filtration through 0.45 μm disposable nylon syringe filters (Membrane-Solutions). 500 μL of the resulting solutions were diluted 1:1 with ultrapure water and analyzed by the developed HPLC method. When further dilution was necessary this was carried out in methanol/water mixtures (50:50 v/v).

2.5. Validation experiments

Validation experiments for the evaluation of the linearity, limits of detection (LOD) and quantification (LOQ), within-day and day-to-day precision and robustness were carried out using the pooled blank matrix mentioned in the previous paragraph. Accuracy studies were performed by spiking individual real wipes samples by

Table 1. Chromatographic figures of merit of the proposed method.

Parameter	MP	EP	PP
Retention time (min)	1.224	2.616	6.668
Peak width (min) [a]	0.08	0.10	0.18
Assymetry factor (A_s)	1.7	1.4	1.1
Resolution factor (R_s)	—	MP/EP = 9.1	EP/PP = 16.8
Plates number (N/m)	25,900	75,825	145,511

[a] *Peak width at half peak height.*

known amounts of a standard parabens mixture. The robustness of the proposed method was examined by deliberate, ± 5% variations in critical LC parameters such as the percentage of the organic modifier and acetic acid in the mobile phase, the flow rate and the column temperature.

3. Results and discussion

3.1. Development of the HPLC method

When developing an HPLC method using short, narrow bore columns some important factors must be considered. Two of these factors are the sample injection volume and the sample solvent [13]. In order to avoid overloading of the column, all samples and standards were injected in 3-μL volumes and to avoid loss in chromatographic efficiency they were dissolved in water / methanol mixtures (1:1) rather than pure organic solvent. A flow rate / temperature combination of 0.4 mL min^{-1} / 40°C was used throughout the study, since it offered acceptable analysis time and backpressures compatible to a conventional LC setup (< 200 bar in all cases).

Hygiene wipes are sample matrices that contain various compounds such as preservatives, fragrances, panthenol, glycerine, citric acid, benzoic acid, phenoxyethanol, PEG-40, hydrogenated castor oil, polysorbate 20, ethoxyl glycerine etc, just to name some. For this reason, the optimization of the mobile phase was not carried out using aqueous standards, but using an extract from a blank pooled matrix of non-parabens containing hygiene wipes. In brief, 1-g pieces from 10 different brands of non-paraben containing wipes were dissolved in 100 mL methanol, extracted for 15 min in an ultrasonic bath and spiked with known amounts of the paraben preservatives (10 mg L^{-1} each). Analysis was performed after 1:1 dilution in water. The experiments proved that the most critical concern was from an early eluting peak that was identified to be phenoxyethanol. The later is present in most of the samples and often co-eluted with methylparaben. After several trials with different mobile phases we concluded to a mixture of acetonitrile / 0.1% acetic acid (20 : 80 v/v). Under the selected conditions, phenoxyethanol and MP were baseline separated (R_s > 1.9), the retention times were reproducible (s_r = 0.3 - 0.4%) and the separation cycle was completed at an acceptable time of < 8 min.

The main chromatographic parameters of the developed HPLC method - including retention times, resolution factors, peak widths and plates numbers per column meter - can be found in Table 1.

3.2. Validation of the proposed method

All analytes were found to obey linearity in the range of 0.5 - 20 mg L^{-1} (n = 8). The respective regression equations and correlation coefficients (r) were:

A_{MP} = 136.85 (± 0.52) × γ(MP) - 5.18 (± 0.94) ,
r = 0.9995

A_{EP} = 121.63 (± 0.23) × γ(EP) - 6.38 (± 1.39) ,
r = 0.9997

A_{PP} = 118.88 (± 0.16) × γ(PP) - 11.94 (± 1.55) ,
r = 0.9993

Where A is the area of the respective peaks and γ is the mass concentration of the analytes in mg L^{-1}. The linearity was further validated by the residuals approach. In all cases, the residuals were distributed randomly along the x-axis and the relative error (e_r) in the back-calculated concentrations was in the range of ± 3%.

The limits of detection (LOD) and quantification (LOQ) of the method were calculated based on the signal-to-noise (S/N) criterion. The noise was evaluated by the Clarity software in the range of 4.0-6.0 min and its value was 0.14 mV. The LODs (S/N = 3) were found to be 15 (MP), 22 (EP) and 43 (PP) μg L^{-1} and the LOQs (S/N = 10), 50 (MP), 73 (EP) and 142 (PP) μg L^{-1}. Taking into account of the sample preparation procedure, the latter values correspond to LODs of 0.30 μg (MP), 0.44 μg (EP) and 0.86 μg (PP) per gram of solid sample and LOQs of 1.0 μg (MP), 1.5 μg (EP) and 2.8 μg (PP) per gram of solid sample respectively.

Table 2. Accuracy of the proposed HPLC method.

Sample	Recoveries (%, at 5 mg L^{-1} level)		
	MP	EP	PP
S1	94.6	96.9	96.8
S2	98.3	96.4	97.1
S3	97.2	96.8	99.1
S4	97.5	103.4	101.9
S5	101.3	96.6	97.2
S6	100.9	98.1	98.3
Mean	98.3 ± 2.5	98.0 ± 2.7	98.4 ± 1.9

Table 3. Analysis of commercially available hygiene wipes by the proposed HPLC method.

Sample	Parabens found ($\mu g\ g^{-1}$)		
	MP	EP	PP
S1	141.5 (± 8.1)	—	28.3 (± 0.6)
S2	146.6 (± 7.3)	—	28.9 (± 0.8)
S3	113.2 (± 7.0)	10.1 (± 0.9)	34.4 (± 1.1)
S4	—	—	77.9 (± 2.3)
S5	101.5 (± 5.3)	—	33.3 (± 1.5)
S6	2342.5 (± 30.9)	—	—

The within-day precision of the method was evaluated by eight repetitive analyses of a blank pooled matrix sample spiked with the three parabens at the 5 mg L^{-1} level. The experimental relative standard deviations (s_r) of the peak areas were 1.3% (MP), 1.4% (EP) and 1.5% (PP). The day-to-day precision was validated by constructing six calibration curves for the three analytes within one working week. The experimental s_r of the slopes was quite satisfactory being 3.7% (MP), 4.6% (EP) and 3.4% (PP).

The accuracy of the analytical procedure was evaluated by spiking several individual samples with parabens standards at the 5 mg L^{-1} level. The experimental results are tabulated in Table 2. As can be seen from the values of the table, the mean percent recoveries were satisfactory, being 98.3% for MP, 98.0% for EP and 98.4% for PP.

As a criterion for robustness we used the resolution factor (R_s) of phenoxyethanol and MP based on the worst case approach. As mentioned in section 3.1, phenoxyethanol is a major ingredient in the real samples and under the selected conditions is adequately separated from MP (R_s > 1.9). During robustness experiments the value of the resolution factor was decreased only at elevated fractions of the organic solvent but remained > 1.5 in all cases, verifying the effectiveness of the procedure.

3.3. Study of the sample treatment

The paraben preservatives were extracted from the wipe samples using methanol as solvent assisted by ultrasonication. The effect of the extraction time was examined in the range of 5 to 30 min. The experiments confirmed the rapid extraction of the analytes in the methanolic solution by obtaining a “steady state” profile in the range of 10-30 min. A reasonable extraction time of 15 min was therefore selected.

Another parameter of the treatment procedure was the effect of a drying process on the recoveries of the analytes, since hygiene wipes typically contain significant amounts of alcohol. In a series of experiments, 1-g pieces of several commercially available samples either were extracted directly, or were dried overnight at 50°C until constant weight was obtained. No statistically significant differences (t-test) were observed between the two groups of samples, indicating no loss of the analytes. The drying process was therefore adopted in all cases due to more convenient handling of the samples.

3.4. Analysis of real samples

Nine Paraben-containing (S1-S6) and paraben-free (S7-S9) commercially available hygiene wipes were analyzed by the proposed method, following the treatment procedure described in the experimental section. The results (in μg parabens per gram of solid samples) are tabulated in Table 3. Absence of parabens in samples S7-S9 was confirmed experimentally (samples not included in Table 3). Representative chromatograms of (a) a paraben-containing wipe and (b) a paraben-free wipe can be seen in Fig. 1.

4. Conclusions

The proposed HPLC method for the determination of three paraben preservatives in commercially available hygiene wipes offers some interesting features: (i) to the best of our knowledge, this is the first application of a core-shell analytical column to this type of separation/analysis; (ii) the developed method is simple and enables the isocratic separation of the analytes in less than 8 min using conventional HPLC instrumentation; (iii) at a low flow rate of 0.4 mL min^{-1}, the consumption of the organic modifier is only 0.64 mL per run; (iv) compared to a recent UPLC method for the determination of MP

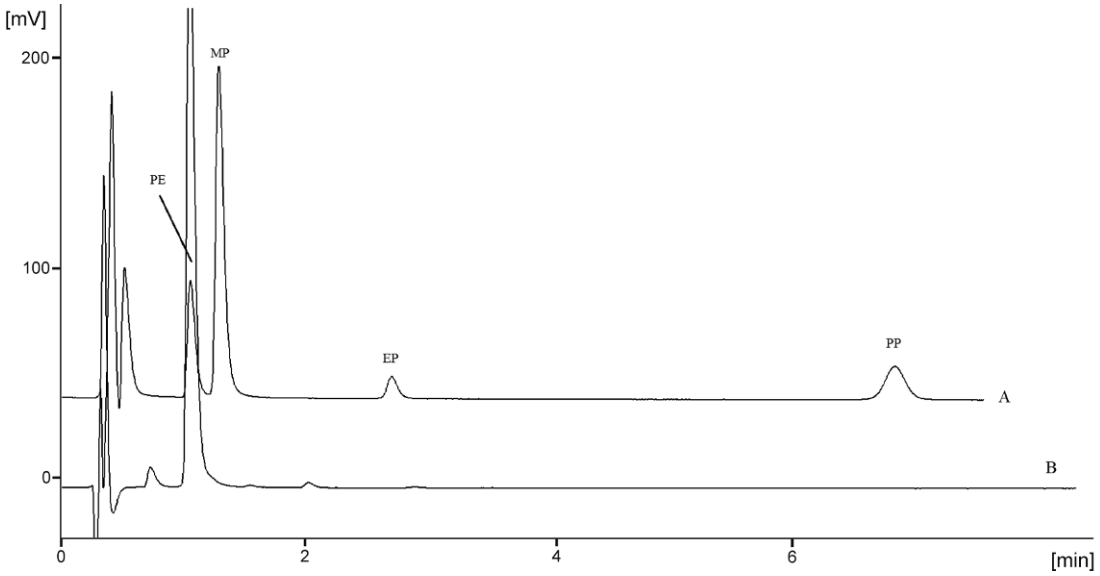

Figure 1. Representative chromatograms of (A) a paraben-containing hygiene wipe, (B) a paraben-free hygiene wipe; PE = phenoxyethanol, MP = methylparaben, EP = ethylparaben, PP = propylparaben.

[10], our method offers comparative retention times and sensitivity using simpler instrumentation and isocratic elution; (v) the method was proved to be suitable for the effective and reliable determination of paraben preservatives in personal hygiene products.

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