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Short communication

Experimental design and capillary electrophoresis for simultaneous analysis of arbutin, kojic acid and hydroquinone in cosmetics

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Abstract

A statistical experimental design was used to optimize one micellar electrokinetic capillary electrophoresis (MEKC) for simultaneous analysis of arbutin (AR), kojic acid (KA) and hydroquinone (HQ). Untreated fused-silica capillaries were operated using a phosphate buffer (20 mM, pH 6.5) under 20 kV and detection at 200 nm. Quantitative linear ranges were 20–200 μg/ml for AR, 20–100 μg/ml for KA and 8–80 μg/ml for HQ with correlation coefficients ≥0.9994. R.S.D. and R.E. were less than 3.0% for the intra-day and inter-day analysis, and all recoveries were greater than 99%. Our method was applied to assay commercial cosmetics. The results were within the labeled amount of 99.6–102.5%.

Keywords: Arbutin; Kojic acid; Hydroquinone; Cosmetics; Experimental design; CE

1. Introduction

Among experimental design, full factorial designs are the most conservative of all design types. Factorial experiments allow experiments to take place over the whole range of the factor space. They show a high degree of precision in exchange for a minimum experimental effort, and they enable factor interactions to be detected [1,2]. In order to increase efficiency of method development, we used a systemic design in establishing one analytical method.

Arbutin (AR), 4-hydroxyphenyl-β-D-glucopyranoside, extracted from leaves of common bearberry, is often used in skin care products as a whitening agent [3]. It can compete with L-dopa for receptor site on tyrosinase and hinders the oxidation of L-dopa, thus, can inhibit the formation of eumelanin [4]. Hydroquinone (HQ), 1,4-benzenediol, was reported by Spencer to be effective at 1.5–2% in a vanishing cream [5]. It can produce reversible depigmentation of the skin by suppression of the melanocyte metabolic processes [6]. Kojic acid (KA), 5-hydroxy-2-(hydroxymethyl)-4H-pyran-4-one, a natural substance produced by fungi or bacteria such as *Aspergillus*, *Penicillium* or *Acetobacter* spp., is also used as a whitening cosmetic because it inhibits tyrosinase. [7]. The Taiwan FDA has announced that the limits of AR and KA as whitening ingredients in cosmetics are 7% and 2%, respectively. It also recommended that HQ is only used under prescription because long-term contact of HQ in concentrations over 5% may cause severe side effects including dermatitis, erythema, leukoderma, burning and hyperpigmentation [8].

According to the literature, some methods such as TLC [4], HPLC [4,9–11], voltammetry [12], spectrophotometry [13] and CE [14,15] have been reported for the analysis of these analytes. Huang et al. [9] used HPLC to analyze these analytes. Among the CE methods, Sakodinskaya et al. [14] used borate buffer for the assay of HQ, Glockl et al. [15] analyzed the AR metabolites in human urine after oral intake of bearberry leaves. There also are some capillary zone electrophoresis (CZE) methods for phenolic compounds in olive oils [16–18], honey [19] and wine [20,21]. None of them have applied CE for the determination of AR, KA and HQ in skin whitening cosmetics. CE yields high-speed, high-resolution, and low consumption of sample and reagents.

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The aim of this study was to develop a rapid, sensitive and quantitative assay for the simultaneous determination of AR, KA and HQ by CE. Optimization of parameters and validation of this method were investigated, and the application of analyzing AR, KA and HQ in cosmetics was also demonstrated.

2. Experimental

2.1. Materials

AR (98%), KA and HQ were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium dihydrogen phosphate, urea and sodium dodecyl sulfate were from Merck (Darmstadt, Germany). All chemicals used were of analytical grade. NeoStrata® pigment lightening gel (2% KA, Princeton, NJ, USA), Shiseido® white lucent (3% AR, Tokyo, Japan), Grape® Quincare ointment (2% HQ, Chung-Li, Taiwan) were used for the applications. Milli-Q water (Millipore, Bedford, MA, USA) was used for preparation of the sample, buffer and related aqueous solutions.

2.2. CE system

A Beckman P/ACE System 2200 (Fullerton, CA, USA) equipped with a filter UV detector and a liquid-cooling device was used. MEKC was performed in an uncoated fused-silica capillary (50 μm I.D., 50 cm effective length and 57 cm total length) (Polymicro Technologies, Phoenix, AZ, USA) using a phosphate buffer (20 mM, pH 6.5), and detected at 200 nm. Required pH value was adjusted with 10 M NaOH. Samples were loaded by pressure injection applying 50 mbar for 5 s. Before start-up, capillary was preconditioned with 0.1 M NaOH solution, water, and running buffer each for 10 min in regular sequence. Between runs, the capillary was rinsed with 0.1 M NaOH solution (5 min) and running buffer (5 min). Electrophoresis was carried out at 25 °C and 20 kV. The current was gradually increased to about 40 μA during the first 15 s after power application. All operations and electropherograms were computer-controlled using GOLD software version.

2.3. Reference and sample solutions

Stock solutions of AR, KA and HQ at 2.0 mg/ml were prepared in water and suitably diluted as reference solutions. Sample solutions were prepared as follows: 47.25 mg of Shiseido® cosmetic, 40 mg of NeoStrata® gel, or 30 mg of Grape® ointment was transferred to three 10-ml volumetric flasks, respectively, and dissolved by water alone. After 30 min of sonication, the suspension was obtained and centrifuged at 1000 × *g* for 10 min. The lower water layer was transferred for analysis.

2.4. Choice of factors and full factorial design

Based on the pK_a values of AR and HQ at about 10 [14] and kojic acid at 7.90 and 8.03 [22], the three can be fully deprotonated in the pH > 11 buffer. Due to rapid oxidation, HQ is unstable in basic media [22]. A lower pH (≤7) was used for ensuring the stability of HQ. Nevertheless, AR and HQ remained neutral, and KA remained partially negative under these conditions. Based on initial experiments, the total migration time will be over 10 min when pH is lower than 5.5, and when higher than 7.0, KA and HQ will migrate together again. Therefore, the pH range of 5.5–7.0 was evaluated in the following study. CZE could not achieve a baseline separation of the analytes, therefore MEKC mode was used. When using SDS (60 mM), AR and HQ could be separated, but HQ and KA migrated together. Optimal concentration of micelle provides sufficient interaction to separate analytes. Hence, the SDS concentration range of 80–120 mM was tested. Meanwhile, buffer concentration (10–25 mM), temperature (20–30 °C) and the applied voltage (15–30 kV) were considered. We observed that temperature and voltage had less influence on separation and were therefore kept constant at 25 °C and 20 kV. As a result, the appropriate selection of the experimental domain was set for buffer concentration, buffer pH and SDS concentration.

In this study, we used a three-level full factorial design to estimate the affect of the three factors for separation, including SDS (80, 100, 120 mM), pH (5.5, 6.5, 7.0), and phosphate buffer (10, 20, 25 mM). The design was carried out in a randomized sequence, and the migration times and peak widths were measured. Resolution (Rs) and migration time were used to assess the optimization of the experimental result.

3. Results and discussion

3.1. Method development

The analysis of coefficients was considered in order to identify the active factors, upon whose alteration a statistically significant variation of the response was observed. The preliminary data show the estimates (coefficients of the linear model found by least squares) of the parameters in the linear model and a *t*-test for the hypothesis that each parameter is zero. The statistically significant coefficients were those where their absolute value was greater than zero with a probability of 95% [23]. From the results we can know that only the concentration of SDS and buffer pH was found to be a significant variable for response function. Buffer concentration has less influence on separation. We also found that when using buffer concentration as 10 mM, poor reproducibility was obtained. This may be due to poor buffer capacity at a lower concentration. To avoid the poor reproducibility, the ideal phosphate buffer concentration was set at 20 mM.

Fig. 1 is the three-dimensional plot of Rs, as a function of buffer pH and SDS concentration. The surface plot allows the whole range of conditions to be explored, including combinations that were not experimentally demonstrated [1]. No distinguishable optimum could be found for the responses within the tested domain. The results indicated that SDS concentration had less influence than the buffer pH. In CE, baseline separation is arrived if Rs ≧ 1.25. While a greater Rs may follow by a longer analysis time, we set an Rs range of 1.25–3 as the optimum domain for experiments. Finally, we selected 20 mM phosphate with pH 6.5, SDS 100 mM to be the optimum elec-

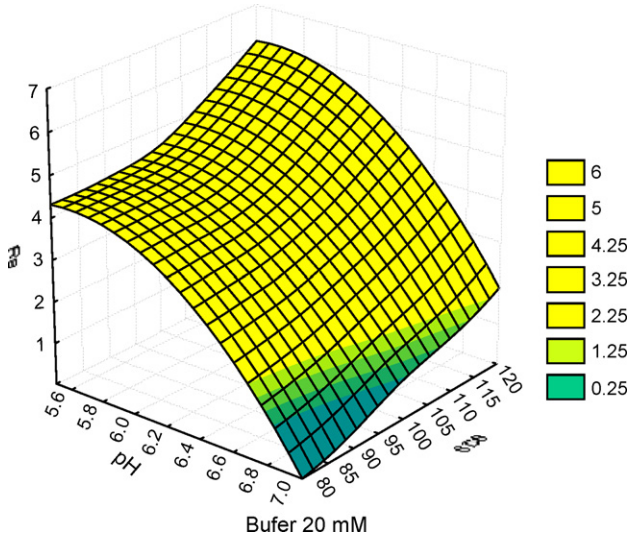

Fig. 1. Graphical representation of the overall resolution (Rs) as a function of pH and SDS concentration.

trophoretic conditions to separate three analytes. Fig. 2(A) is the composite electropherograms of reference standards and blank under these conditions. The total separation was ended within 6 min, which is much quicker than that of one published HPLC method (18 min) [9]. Using these conditions a method validation study was performed.

3.2. Method validation

To evaluate the quantitative applicability of this method, five different concentrations of AR, KA and HQ, in the range of 20–200 μg/ml, 20–100 μg/ml and 8–80 μg/ml, respectively, were analyzed using urea (4 mg/ml) as an internal standard (IS). Linearity between the normalized peak-area ratios (Y) of the related analyte to IS and the concentration (X, mg/ml) of analyte was investigated. The regression equations of intra- and inter-day analysis were calculated from the assay values of prepared standard triplicates on a single day ($n = 3$) and on 5 consecutive days ($n = 5$). As shown in Table 1, results of the regression equations indicate that high linearity ($r \geq 0.9994$) between Y and X was attained over the range studied. The detection limits (S/N = 3, injection 5 s at 50 mbar) were 5.4 μg/ml (AR), 7.1 μg/ml (KA) and 2.2 μg/ml (HQ). For greater precision and accuracy in evaluation, the relative standard deviation (RSD) and relative error (RE) of the method, based on statistical determination ($n = 3$) of each analyte at three different concentrations (μg/ml) (AR: 50, 90, 160; KA: 30, 70, 90; HQ: 15, 30, 70) were studied (data not shown). The RSD and RE were less than 3.0%,

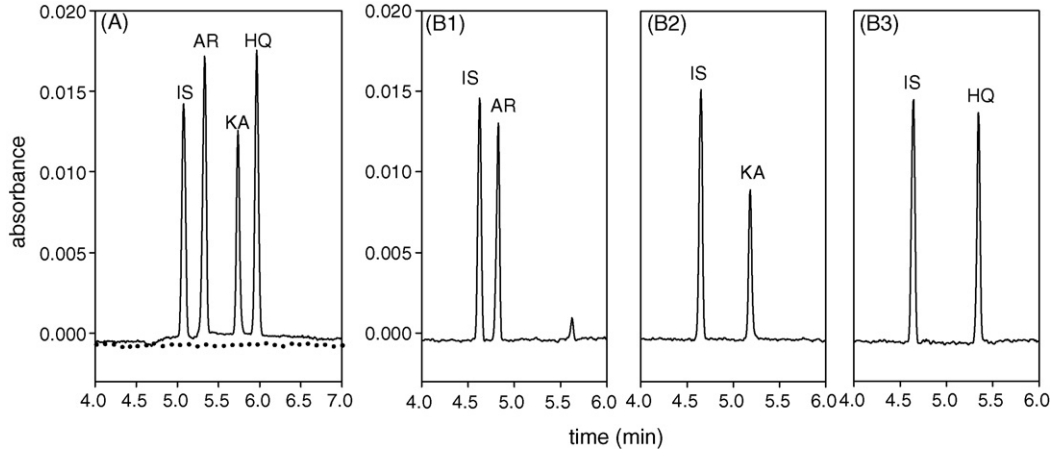

Fig. 2. Electropherograms of (A) standards (solid line) and blank(dotted line), (B) commercial cosmetics: (B1) Shiseido® white lucent; (B2) Grape® Quincare ointment; (B3) NeoStrata® pigment lightening gel. CE conditions: phosphate buffer (20 mM, pH 6.5) containing SDS 100 mM; 20 kV; uncoated fused-silica capillary, 50 cm (effective length) × 50 μm ID; injection 5 s at 50 mbar; 200 nm. Peaks: AR, arbutin; KA, kojic acid; HQ, hydroquinone; IS, internal standard, urea.

Table 1
Regression analysis for the determination of AR, KA and HQ

Analysis	Regression equation	Coefficient of correlation (r)
Intra-day[a]		
AR	$Y = (5.23 \pm 0.02)X + (0.015 \pm 0.002)$	0.9998
KA	$Y = (6.09 \pm 0.03)X + (0.002 \pm 0.005)$	0.9994
HQ	$Y = (11.47 \pm 0.02)X + (0.012 \pm 0.003)$	0.9998
Inter-day[b]		
AR	$Y = (5.23 \pm 0.05)X + (0.011 \pm 0.005)$	0.9997
KA	$Y = (6.07 \pm 0.12)X + (0.002 \pm 0.005)$	0.9996
HQ	$Y = (11.39 \pm 0.11)X + (0.001 \pm 0.007)$	0.9998

Concentration ranges for the intra- and inter-day analysis: arbutin: 0.02–0.2 mg/ml; kojic acid: 0.02–0.1 mg/ml; hydroquinone: 0.008–0.08 mg/ml.
[a] The regression equations of intra-day analysis were calculated from the assay values of prepared standards on a single day ($n = 3$).
[b] The regression equations of inter-day analysis were calculated from the assay values of prepared standards on five days ($n = 5$).

and the recoveries were greater than 99% (data not shown). Some common whitening agents, antioxidants and preservatives were examined, and did not show any interference. These agents were ascorbic acid, ascorbic acid-6-palmitate, L-ascorbic acid-2-phosphate sesquimagnesium salt, dodecyl gallate, 2,6-di-*tert*-butyl-4-methylphenol, and DL-α-tocopherol.

3.3. Applications

Applications of the method to the assay of AR, KA and HQ in three commercial cosmetics were studied. The individual electropherograms are shown in Fig. 2, and presented no interference from real samples. The contents of each tested cosmetics were 2.99–3.01% AR for Shiseido® cosmetics, 2.01–2.05% KA for Grape® ointment, and 1.99–2.03% HQ for NeoStrata® gel. The contents of these cosmetics also correspond to the limits requested by the Taiwan FDA, which are 7% AR, and 2% KA. Besides, HQ fell within the labeled amount of 94–106% required by the USP XXV [13].

4. Conclusions

Combining experimental design, we narrowed down the parameters investigation and established the MEKC method for the simultaneous determination of AR, KA and HQ, and successfully applied this to quality control of skin whitening cosmetics.

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References

[1] M.E. Capella-Peiro', A. Bossi, J. Esteve-Romero, Anal. Biochem. 352 (2006) 41–49.
[2] H. Brunnkvist, B. Karlberg, A. Astervik, I. Granelli, J. Chromatogr. B 807 (2004) 293–300.
[3] I. Parejo, F. Viladomat, J. Bastida, C. Codina, Phytochem. Anal. 12 (2001) 336–339.
[4] M.O. Masse, V. Duvallet, M. Borremans, L. Goeyens, Int. J. Cosmet. Sci. 23 (2001) 219–232.
[5] M.C. Spencer, Arch. Dermatol. 84 (1961) 131–134.
[6] C.F. Lacy, L.L. Armstrong, M.P. Goldman, L.L. Lance, Drug Information Handbook, 11th ed., Lexi-Comp Inc., Canada, 2003, pp. 704–705.
[7] Y. Hasebe, K. Oshima, O. Takise, S. Uchiyama, Talanta 42 (1995) 2079–2085.
[8] Department of Health, Executive Yuan, Ordinance No. 89028104, Taipei, 2000.
[9] S.C. Huang, C.C. Lin, M.C. Huang, K.C. Wen, J. Food Drug Anal. 12 (2004) 13–18.
[10] Y. Shih, J. AOAC Int. 84 (2001) 1045–1049.
[11] M.L. Chang, C.M. Chang, J. Pharm. Biomed. Anal. 33 (2003) 617–626.
[12] Y. Shih, J.M. Zen, Electroanalysis 11 (1999) 229–233.
[13] United States Pharmacopeia 25th ed., National Formulary 20th ed., Asian ed., 2002, pp. 867–868.
[14] I.K. Sakodinskaya, C. Desiderio, A. Nardi, S. Fanali, J. Chromatogr. 596 (1992) 95–100.
[15] I. Glockl, G. Blaschke, M. Veit, J. Chromatogr. B 761 (2001) 261–266.
[16] A. Carrasco-Pancorbo, A.M. Gomez-Caravaca, L. Cerretani, A. Bendini, A. Segura-Carretero, A. Fernandez-Gutierrez, J. Agric. Food Chem. 54 (2006) 7984–7991.
[17] A. Carrasco-Pancorbo, C. Cruces-Blanco, A. Segura-Carretero, A. Fernandez-Gutierrez, J. Agric. Food Chem. 52 (2004) 6687–6693.
[18] M. Bonoli, M. Montanucci, T.G. Toschi, G. Lercker, J. Chromatogr. A 1011 (2003) 163–172.
[19] A.M. Gomez-Caravaca, M. Gomez-Romero, D. Arraez-Roman, A. Segura-Carretero, A. Fernandez-Gutierrez, J. Pharm. Biomed. Anal. 41 (2006) 1220–1234.
[20] Z. Guadalupe, A. Soldevilla, M.P. Saenz-Navajas, B. Ayestaran, J. Chromatogr. A 1112 (2006) 112–120.
[21] P.B. Andrade, B.M. Oliveira, R.M. Seabra, M.A. Ferreira, F. Ferreres, C. Garcia-Viguera, Electrophoresis 22 (2001) 1568–1572.
[22] S. Budavari, The Merck Index, 12th ed., Merck, Rahway, NJ, 1996, p. 5330.
[23] Y.L. Loukasa, S. Sabbah, G.K.E. Scriba, J. Chromatogr. A 931 (2001) 141–152.