Comparison of RP, Ion-Pair and IE Chromatography Coupled with UV and Tandem Mass Spectrometry for the Determination of 8-Hydroxyquinoline in Cosmetics

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Abstract

Liquid chromatographic methods based on reversed-phase, ion-pair and ion-exchange chromatography coupled with ultraviolet spectrometry and tandem mass spectrometry have been developed and compared for the determination of 8-hydroxyquinoline in cosmetic samples. The individual parameters of different techniques are systematically optimized. The reversed-phase chromatography using ultrasonic extraction, centrifugation and filtration prior to LC–UV and LC–MS–MS detection was selected to be the general analytical method for the routine analysis of 8-hydroxyquinoline in cosmetics due to its simplicity, column reproducibility and sensitivity. The procedure is rapid, simple and suitable for the quality control of cosmetic preparations. The application of this technique to the assay of commercial products is also presented.

Keywords

Column liquid chromatography
Ion-pair chromatography
Ion-exchange chromatography
8-Hydroxyquinoline in cosmetics

Introduction

8-Hydroxyquinoline, known as a mono-protic bidentate chelating agent, is extensively used as the antiseptic, disinfectant and deodorant in the production

and preservation of cosmetic products for its strong antimicrobial property [1]. 8-Hydroxyquinoline has demonstrated some oncogenic potential when implanted in the mouse bladder in a cholesterol vehicle and when instilled in

the rat vagina [2, 3]. Since cosmetics are widely used consumer goods, the related regulations, as their prime and principal purpose, highly concern the guaranteeing of consumer safety. According to the European Union Council Directive 76/768/EEC, 8-hydroxyquinoline is listed as the substance which must not form part of the composition of cosmetic products except for the uses under maximum authorized concentration restrictions of 0.3% (w/w) in rinse-off hair-care preparations and 0.03% (w/w) in non-rinse-off hair-care preparations [4]. Hence, the assay of 8-hydroxyquinoline in commercial cosmetic products is important for quality control purposes and for checking their conformance to the current legislation.

The widely used methods for the determination of 8-hydroxyquinoline are titrimetry [5], polarography [6], colorimetry [7, 8], spectrophotometry [9], thin layer chromatography (TLC) [10], and liquid chromatography (LC) [11–13]. However, a detailed study of these publications would indicate that improvement is in great need, since there are no analytical methods specifically for the analysis of 8-hydroxyquinoline in cosmetics so far. It is also necessary to provide the sample pretreatment and

instrumentation protocol with greater specificity and sensitivity.

In this study, the analytical methods based on reversed-phase, ion-pair and ion-exchange chromatography coupled with ultraviolet spectrometry and tandem mass spectrometry were established, and the chromatographic behaviors in different liquid chromatographic modes were compared. The proposed methods have been successfully applied to determine the content of 8-hydroxyquinoline in cosmetic products, and is applicable to the quality control process of cosmetics to verify their adherence to relevant legislation.

Experimental

Reagents and Materials

Organic solvents such as methanol, acetonitrile and tetrahydrofuran of LC grade were from Fisher (Pittsburgh, PA, USA). 8-Hydroxyquinoline of 99% purity was obtained from Chem Service (West Chester, PA, USA). Sodium heptyl sulfate was from J.T. Baker (Phillipsburg, NJ, USA). Sodium hexyl sulfate and sodium decyl sulfate were purchased from Dikma (Lake Forest, CA, USA). Trifluoroacetic acid (TFA) was from Tedia (Fairfield, OH, USA), phosphoric acid and potassium dihydrogen phosphate were obtained from Alfa Aesar (Ward Hill, MA, USA). All water used in this study was prepared with a Milli-Q water purification system (Millipore, Bedford, MA, USA) under a resistivity of $18.2 \text{ M}\Omega \text{ cm}^{-1}$. The pH meter was from Mettler Toledo (Columbus, OH, USA).

Sample Collection and Preparation

A variety of untreated cosmetic samples of different categories (lotion, cream, liquid, gel, powder, shampoo, etc.) were collected from the cosmetic industries of China, France, United State, Germany, Italy, Japan, Korea, etc. The origins of all of the cosmetic samples were known before analysis. About 0.5 g of the cosmetic sample was placed into a glass

tube, and 10 mL of methanol was added. The glass tube was shaken on a vortex mixer for about 30 s, and then extracted under sonication for 20 min. After centrifuged at 12,000 rpm for 10 min, the clear supernatant was filtered by $0.45 \mu\text{m}$ membrane before injection for instrumental analysis.

Instrument and Chromatography

Liquid chromatography was performed on a 1200 LC system (Agilent Technologies, Palo Alto, CA, USA) equipped with online degasser, quaternary pump, autosampler with 100 μL injection loop, thermostatic compartment, and diode array detector. Analysis of 8-hydroxyquinoline was carried out based on different liquid chromatographic separation mechanism, i.e. reversed-phase, ion-pair and ion-exchange chromatography respectively, with the detection wavelength of 240 nm for optimum sensitivity.

The separation of 8-hydroxyquinoline in the reversed-phase chromatography was conducted with a Waters XTerra MS C_{18} chromatographic column ($4.6 \text{ mm id} \times 250 \text{ mm length}$, $5 \mu\text{m}$ particle size). The mobile phase for eluting the analyte comprised 20% A (0.1% trifluoroacetic acid in methanol) and 80% B (0.1% trifluoroacetic acid in water) at a flow-rate of 1.0 mL min^{-1} . The ion-pair chromatography was performed on a chromatographic column packed with Kromasil C_{18} ($4.6 \text{ mm id} \times 250 \text{ mm length}$, $5 \mu\text{m}$ particle size) under isocratic elution with the mobile phase of 55% A (consisting of 10 mmol L^{-1} potassium dihydrogen phosphate and 20 mmol L^{-1} sodium heptyl sulfate, adjusted to pH 2.25 with orthophosphoric acid) and 45% B (methanol) at a flow-rate of 1.0 mL min^{-1} . The ion-exchange chromatography was carried out on a Waters Spherisorb SCX chromatographic column ($4.6 \text{ mm id} \times 250 \text{ mm length}$, $5 \mu\text{m}$ particle size) under isocratic elution with the mobile phase composed of 55% A (150 mmol L^{-1} potassium dihydrogen phosphate, adjusted to pH 2.25 with orthophosphoric acid) and 45% B (methanol) at a

flow-rate of 1.0 mL min^{-1} . In all the three methods, the column was thermostated at 35°C and the UV detection at wavelength 240 nm was used for the determination of 8-hydroxyquinoline. The chromatographic parameters of 8-hydroxyquinoline in the reversed-phase, ion-pair and ion-exchange chromatography are shown in Table 1.

Mass Spectrometry

A Quattro micro atmospheric pressure ionization (API) mass spectrometer (Micromass, Manchester, UK) equipped with an ESI source was used. The Z-Spray dual orthogonal sampling interface allowed the entire column effluent from the liquid chromatograph to be directed into the MS source without flow splitting, which contributed to the greatly enhanced sensitivity and ruggedness. Typical ion source parameters were set as follows: ESI capillary voltage 3.5 kV, cone voltage 35 V, extractor voltage 5 V, RF lens voltage 0.5 V, source block temperature 120°C , desolvation temperature 350°C , nitrogen desolvation gas flow rate 500 L h^{-1} , cone gas flow rate 50 L h^{-1} , ion energy 1 and 2 0.5 V, and multiplier voltage 650 V. On the occasion of the presence of 8-hydroxyquinoline in the real cosmetic samples detected by liquid chromatography, it was necessary to conduct LC-MS-MS analysis for confirmation. Qualitative analysis was performed in multiple reaction monitoring (MRM) mode under positive ion conditions, in which the analyte was first detected as protonated molecular ion of 145.9, and then fragmented in the MS-MS mode to produce the product ions of 117.6 and 127.7 with the collision energy of 18 and 23 eV, respectively, to achieve four identification points complying with the European Union Directive 2002/657/EC [14].

Results and Discussion

Silanol Effect

8-Hydroxyquinoline is an amphoteric compound containing two ionizable functional groups individually capable

Table 1. Chromatographic parameters for 8-hydroxyquinoline in the reversed-phase chromatography, ion-pair chromatography and ion-exchange chromatography

Parameters	Reversed-phase chromatography	Ion-pair chromatography	Ion-exchange chromatography
Chromatographic column	XTerra MS C ₁₈ column (250 mm × 4.6 mm, 5 μm)	Kromasil C ₁₈ column (250 mm × 4.6 mm, 5 μm)	Spherisorb SCX column (250 mm × 4.6 mm, 5 μm)
Column temperature (°C)	30	30	30
Mobile phase	20% A (0.1% TFA in methanol) and 80% B (0.1% TFA in water)	55% A (10 mmol L ⁻¹ potassium dihydrogen phosphate + 20 mmol L ⁻¹ sodium heptyl sulfate, pH 2.25 adjusted with orthophosphoric acid) and 45% B (methanol)	55% A (150 mmol L ⁻¹ potassium dihydrogen phosphate, pH 2.25 adjusted with orthophosphoric acid) and 45% B (methanol)
Flow rate (mL min ⁻¹)	1.0	1.0	1.0
Detection wavelength (nm)	240	240	240
Injection volume (μL)	10	10	10

of acidic or basic behavior with the dissociation constants of $pK_1 = 5.0$ and $pK_2 = 9.9$, respectively [15, 16]. The chromatography concerning the amphoteric compound is very complicated due to several concurrent retention mechanisms which may possibly govern the chromatographic behavior simultaneously, including ion-exchange, hydrophobic interaction, hydrogen-bond interaction, etc. 8-Hydroxyquinoline, as an ionic compound, may interact with the residual silanols inside the silica-based columns in liquid chromatography, and therefore lead to the phenomena of excessive retention, severe band tailing and column-to-column irreproducibility because of silanophilic interactions. It is generally desirable to minimize the silanol interaction effect by adopting appropriate experimental conditions, such as adjusting mobile-phase pH to restrain residue silanols activity or creating the environment containing ion-pair agent to control silanol effect.

Optimization of the Reversed-Phase Chromatographic Parameters

In reversed-phase chromatography, the resulting surface charge of stationary phase mainly depends on the number of positively charged sites and the number of negatively charged sites and is therefore a function of solution pH. The influence of silanol group on the chromatographic behavior of the sample may be suppressed either by using a low-pH

mobile phase to minimize the concentration of ionized silanols on the column surface and to reduce the ion-exchange interactions with the solute or by using a high-pH mobile phase to keep the analyte in an uncharged form and to inhibit the ion-exchange ability of residual silanols toward the protonated analyte. As a result, the selection of proper buffer pH is necessary to reproducibly separate ionizable compounds to achieve a sharp and symmetrical peak. In this study, the pH ranges from 1.5 to 11.0 with a pH-stable column and a series of buffer solutions corresponding to the target pH: pH 1.5 (0.1% trifluoroacetic acid); pH 2.0, 2.25 and 3.0 (10 mmol L⁻¹ potassium dihydrogen phosphate); pH 3.5, 4.0 and 4.5 (10 mmol L⁻¹ ammonium formate); pH 5.0 and 5.5 (10 mmol L⁻¹ ammonium acetate); pH 6.0, 6.5, 7.0, 7.5 and 8.0 (10 mmol L⁻¹ potassium hydrogen phosphate); pH 9.0, 10.0 and 11.0 (10 mmol L⁻¹ ammonium bicarbonate) were investigated. The mobile-phase pH was adjusted to the target pH before adding organic solvent. The results showed that pH was critical for 8-hydroxyquinoline separation and the best chromatographic peak shape and acceptable retention was found at pH 2.25. On the other hand, the chromatographic performance at high pH was far from satisfactory and resulted in bad peak shape. Choice of chromatographic column type including C₁₈, C₈, phenyl, cyano, etc., was studied in order to obtain the optimum performance. Excellent result was exhibited by using a Waters XTerra MS C₁₈ chromato-

graphic column (4.6 mm id × 250 mm length, 5 μm particle size). In terms of solvent type, the three most widely used organic mobile phase, i.e. methanol, acetonitrile and tetrahydrofuran, were observed to show similar chromatographic performance in respective proper proportion to aqueous mobile phase. Methanol was finally chosen to be the first choice because of its relatively lower toxicity, price and greater compatibility for buffers compared to mobile phase containing acetonitrile or tetrahydrofuran. The volume fraction of methanol in the aqueous-organic mobile phase was optimized to obtain the suitable capacity factor under isocratic conditions in a binary mobile phase. It was reported in some literatures [17, 18] that the addition of amine modifiers is beneficial for the chromatographic separation of basic samples due to the blockage of ionized silanols by the amine. In our study, triethylamine and hexylamine, as the common amine modifiers, were tested to observe whether there was even better performance. However, no improved chromatographic behavior of 8-hydroxyquinoline was found. Furthermore, amine modifiers were out of consideration because they can cause slow column equilibration when changing the mobile phase. Silanol effect may also be reduced by choosing a buffer cation which can be held more strongly by the silanols than the analyte and therefore block unnecessary sample retention by the ionized silanols. Buffers in the potassium form normally have strong affinity to silanols and good

solubility in aqueous-organic mobile phase. Taking into account these facts, potassium dihydrogen phosphate was finally chosen to be used in our system, and different buffer concentrations in the mobile phase were optimized. Temperature generally has a much more noticeable effect for the reversed-phase separation of ionic samples than neutral samples, since several retention-related interactions can be involved, each corresponding differently to the changes in temperature. A column temperature of 30 °C was optimized for sample analysis.

Optimization of the Ion-Pair Chromatographic Parameters

In the ion-pair chromatographic mode for the analysis of 8-hydroxyquinoline, the negative ion-pair reagent added is attached to the stationary phase by hydrophobic interaction with alkyl moiety, leaving the stationary phase carried by the negative charged group (e.g., $\text{C}_9\text{-SO}_3^-$), which is further balanced by positive ions (e.g., Na^+) from the ion-pair reagent and positive ions (e.g., K^+) from the buffer. The protonated and positively charged 8-hydroxyquinoline can now exchange with the above Na^+ and K^+ ions, resulting in the retention of 8-hydroxyquinoline by an ion-exchange process. A series of chromatographic parameters, such as ion-pair reagent type and concentration, pH value, buffer type and concentration, solvent type and strength, column temperature were evaluated. In view of the basic property of 8-hydroxyquinoline, several commonly used negative ion-pair reagents, i.e., sodium hexyl sulfate, sodium heptyl sulfate, sodium decyl sulfate were investigated. Sodium heptyl sulfate was shown to have relatively higher efficiency in decreasing solute silanophilic interactions and relatively shorter column equilibration duration due to its moderate hydrophobicity. The presence of sodium heptyl sulfate as the ion-pair reagent significantly increased the retention time of 8-hydroxyquinoline. The mobile phase contains a large number of negatively charged species (SO_3^-) which compete for the analyte and block active,

strongly interacting sites on the surface of silica. A reasonable sodium heptyl sulfate concentration of 20 mmol L^{-1} was chosen in the mobile phase according to retention versus reagent concentration curve to achieve the significant retention of the analyte on the column. In the case of the interaction between 8-hydroxyquinoline and sodium heptyl sulfate, low pH is preferable due to the occurrence of maximum retention as well as the contribution to silanol effect suppression. A pH of 2.25 was found to be the optimum and 10 mmol L^{-1} potassium dihydrogen phosphate buffer with proper buffering range was correspondingly adopted. It is rather important to maintain a constant 30 °C column temperature by thermostat compartment to stabilize several equilibrium processes, such as sample retention by ion-exchange, sorption of the ion-pair reagent on the chromatographic column, reversed-phase processes, etc. When ion-pair chromatography was used, the column was conditioned by passing the mobile phase through it for at least 2 h at a flow-rate of 1 mL min^{-1} .

Optimization of the Ion-Exchange Chromatographic Parameters

Ion-exchange is one of the important retention mechanisms for amphoteric compounds in liquid chromatography. Since there is much similarity between ion-exchange and ion-pair chromatography in terms of retention behavior, many separations that are possibly using ion-exchange chromatography can also be achieved using ion-pair chromatography. In the current study, a cation-exchange column with negative sulfonate group covalently embedded in the stationary phase is utilized for the assay of 8-hydroxyquinoline. Various chromatographic parameters, including buffer type and concentration, column temperature, mobile phase composition, etc., were optimized in the similar manner to reversed-phase chromatographic and ion-pair chromatographic analysis of 8-hydroxyquinoline.

Comparison of the Reversed-Phase, Ion-Pair and Ion-Exchange Chromatography

Chromatographic parameters of reversed-phase chromatography, ion-pair chromatography and ion-exchange chromatography were summarized in Table 1. Results of some typical system suitability data in the three chromatographic modes are listed in Table 2.

In the reversed-phase, ion-pair as well as ion-exchange chromatography, acceptable retention of 8-hydroxyquinoline can be achieved by adjusting the chromatographic parameters. Under the preconditions that all the chromatographic columns utilized in the three chromatographic modes were newly coming into use and were specifically for the analysis of 8-hydroxyquinoline, ion-exchange chromatography exhibited the best peak symmetry, i.e. highest symmetry factor and lowest USP tailing factor. Ion-pair chromatography, in terms of column efficiency for the assay of 8-hydroxyquinoline, was shown to possess the best performance. And reversed-phase chromatography obtained the best repeatability according to the data of RSD of peak area and retention time of 8-hydroxyquinoline. As a result, there is no such a chromatographic mode that has shown dominating advantages over the others and each of them has their own features in certain aspects.

For regular ionic samples, we have a choice of three LC methods: reversed-phase, ion-pair, or ion-exchange chromatography. Because of its simplicity, freedom from problems, and better column reproducibility, reversed-phase chromatography usually is the best starting point for the analysis. Ion-pair chromatography is a logical follow-up to reversed-phase chromatography for the separation of ionic samples, differing mainly in the addition of an ion-pair reagent to the mobile phase. Ion-exchange chromatography is also an alternative method for the analysis of basic compounds. Reversed-phase chromatography and ion-exchange chromatography with a volatile buffer may be compatible with a mass spectrometer

detector, whereas the ion-pair reagent used in this study for ion-pair chromatography is not sufficiently volatile and is not applicable to liquid chromatography coupled with mass spectrometer detection. Generally speaking, reversed-phase chromatography was selected to be the general method for the routine analysis of 8-hydroxyquinoline of the three chromatographic methods, possessing the advantages of column performance, operation simplicity, lower cost, MS compatibility, etc.

Optimization of LC-MS-MS Parameters

Given that non-volatile salt should be avoided and the volatile additive concentration should be kept low enough in order to prevent ion source contamination, ammonium acetate was finally chosen in our system. A series of mass spectrometric parameters, including ESI capillary voltage, cone voltage, extractor voltage, RF lens voltage, collision energy, etc., were optimized detailedly. For the detection by LC-MS-MS, identification occurs with the retention time and diagnostic ions consisting of the daughter ions at m/z 117.6 and 127.7. For confirmatory purpose, a comparison with quality control samples is made using acceptable deviations of $\pm 2.5\%$ for retention time and of $\pm 20\%$ for the ionic relative abundance as described in the European Commission Directive 2002/657/EC laying down performance criteria of analytical methods.

Method Validation

The established reversed-phase chromatographic method was validated for recovery, limit of detection (LOD), linear dynamic range, precision and reproducibility. Recoveries of spiked compound at three levels were between 93.2 and 100.8%. LOD based on injection of a 10 μ L aliquot, calculated as a signal-to-noise (S/N) ratio of three, was 1 ng. The linear dynamic range of the reversed-phase chromatographic instrumentation was evaluated by analyzing 8-hydroxyquinoline standard solutions

Table 2. System suitability data for 8-hydroxyquinoline in the reversed-phase, ion-pair and ion-exchange chromatography

Property	Reversed-phase chromatography	Ion-pair chromatography	Ion-exchange chromatography
t_R (min)	9.275	8.607	9.502
Capacity factor	2.508	2.255	2.594
Symmetry factor	0.717	0.662	0.789
USP tailing factor	1.328	1.466	1.201
PWHM	0.197	0.160	0.189
Plate	12,334	16,001	13,960
Repeatability, A (RSD, %)	0.62	0.97	0.85
Repeatability, t_R (RSD, %)	0.02	0.05	0.04

at the following injected amounts: 1, 5, 10, 50, 100, 500 ng. Calibration curve was generated using linear regression analysis and gave good fit, with the correlation coefficients of 0.9999 over the established range. The precision of the method was determined from the extraction and analyses of triplicate samples. Over the concentration range, within- and between-day precision were found to be 2.15 and 3.12%, respectively. The reproducibility of the method was determined from triplicate analyses of spiked cosmetic samples. The overall reproducibility of the analyses was satisfactory, with the relative standard deviation (RSD) value of 3.68%.

Analysis of Cosmetic Samples

A variety of cosmetic samples of different categories (lotion, cream, liquid, gel, powder, shampoo, etc.) were analyzed by the proposed reversed-phase chromatographic method. From our investigation, there are two hair-care samples found containing 8-hydroxyquinoline with both concentrations lower than 0.03% of the 30 cosmetic samples tested. The two cosmetic samples were subjected to LC-MS-MS confirmatory test to verify the accuracy of the detection.

Conclusions

This paper describes and compares a set of LC methods using different techniques, i.e. reversed-phase, ion-pair and ion-exchange chromatography for the determination of 8-hydroxyquinoline in cosmetic samples. The chromatographic parameters in the three modes and

LC-MS-MS parameters were systematically optimized. The reversed-phase chromatography based on ultrasonic extraction, centrifugation and filtration prior to LC-UV and LC-MS-MS was selected to be the general analytical method for the routine screening analyses of 8-hydroxyquinoline in cosmetics due to its simplicity, column reproducibility and sensitivity, and is suitable for the quality control assay of 8-hydroxyquinoline in cosmetic products.

To our knowledge, this is the first report of the analytical method for the determination of 8-hydroxyquinoline in cosmetic samples, and our measurements provide some scientific data about the presence and dosage of 8-hydroxyquinoline in real cosmetic products of different categories and production origins.

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