Determination of Residual Acrylamide in Medical Polyacrylamide Hydrogel by High Performance Liquid Chromatography tandem Mass Spectroscopy

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Objective To determine residual acrylamide in medical polyacrylamide hydrogel by high performance liquid chromatography tandem mass spectroscopy (HPLC-MS). **Methods** After $^{13}C_3$ labeled acrylamide was added, the sample was extracted with water and then cleaned up with Extrelut™ 20. The polyacrylamide hydrogel sample and 20 clinical cases were analyzed by HPLC-MS/MS and isotope dilution quantifying technique in selected reaction monitoring (SRM) mode. **Results** Acrylamide was separated from polyacrylamide hydrogel. The concentration of acrylamide in polyacrylamide hydrogel ranged from 3.9×10^{-9} to 3.1×10^{-8}g/L in the 20 clinical cases. The peak area was favorable linear and the range was up to 3 000 μg/L. The recovery rate was 103.1% with a relative standard deviation (RSD) of 6.20%, when the mark level was 50 μg/L. **Conclusion** HPLC-MS is a rapid, accurate, and sensitive method for the determination of residual acrylamide in medical polyacrylamide hydrogel.

Key words: High performance liquid chromatography-mass spectroscopy; Polyacrylamide hydrogel; Acrylamide

INTRODUCTION

Polyacrylamide hydrogel (PAHG), a polymer of controllable molecular weight formed by the polymerization of acrylamide monomers (AM), has been widely used in food, water supply, and smelting industry as a medical material or a permanent filling material for more than 15 years in Ukraine and Russia and for 9 years in China and Eastern European countries. It was forbidden to use by the State Drug and Food Administration of China in 2006. In China, there are about 300 000 patients undergoing plastic surgery, most of whom have received injection for breast augmentation. However, major concern has been widely expressed because AM used to produce PAHG is implicated as a potential mutagen and reproductive toxicant[1]. The gel specimen is transparent with an optimum viscosity, but during operation the gel taken out of the injection position has become primrose yellow and has a low viscosity with granules. It is, therefore, necessary to investigate the toxicity of residual monomer in medical PAHG since contamination of the AM cannot be totally avoided in the manufacturing process and the stability of PAHG in the human body is unknown.

Several methods for AM determination are available, such as spectrometry and high performance liquid chromatography[2]. However, majority of these methods are not so accurate and is vulnerable to potential interference. In this paper, a practical and simple method for determining AM, especially in PAHG, is presented. The limit of detection for AM can be achieved in the ppb range. We extracted AM in PAHG from 20 patients who had undergone the injection of PAHG for breast augmentation, which was detected by high performance liquid chromatography tandem mass spectroscopy (HPLC-MS)[3-4]. The results indicate that HPLC-MS is accurate in determining AM in PAHG.

MATERIALS AND METHODS

Reagents and Chemicals

The following materials and reagents were used: AM (>99.8%, suitable for electrophoresis, Fluka),

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$^{13}C_3$ labeled AM (Cambridge Isotope Laboratories), methanol (HPLC grade, J&T BAKER), extra pure water (18. 2 MΩ), methanoic acid (analytical reagent, Beijing Chemical Regents Co.), methanoic acid (chromatographically pure, Dikma), hexane (analytical reagent, Beijing Chemical Regents Co.), ethyl acetate (analytical reagent, Beijing Chemical Regents Co.), ammonium sulfate (analytical reagent, Beijing Chemical Regents Co.), sodium sulfate anhydrous (analytical reagent, Beijing Chemical Regents Co.), industrial nitrogen (>99.5%, concentrated), extra pure helium (>99.995%, LC-MS pro analysis collision gas), liquid nitrogen (LC-MS pro-analysis dissolving gas) and Extrelut™ 20 diatomite (Merck, Germany).

The specimen of PAHG was the gel taken from the 20 women during the operation in 2007 who were undergoing injection of PAHG for breast augmentation. The standard sample of PAHG was amazing gel (from Fu Hua Co., Ltd., lot: 20040105MJ01).

Apparatus

The HPLC system (Surveyor, Thermo Finnigan) and MS equipments were used in experiments. HPLC-MS interface on a thermo finnigan LTQ linear iontrap mass spectrometer was used to acquire the HPLC-MS data. HPLC separation was performed on MG120 C18 column (150 mm×4.6 mm, 5 μm particles, I. D. SHISEIDO, Japan) using a mobile phase of methanol and 0.1% methanoic acid (1：9, v/ v) at a flow rate of 0.6 mL/min in a column oven at 26 ℃ and 10 μL sample. The mass spectra were obtained in a full-scan mode. Measurement of the detection limits and quantification were performed by selected reaction monitoring (SRM) for the base ion peaks of AM (m/z 72→55) and $^{13}C_3$ AM (m/z 75→58). Cation atmospheric pressure chemical ionization (APCI+), discharge current 5 uA, capillary temperature of 300 ℃, sheath gas (N2) of 30Arb, aided gas (N2) of 10 Arb, collision-induced dissociation (CID) at 5 V, activiation Q of 0.5 and collision energy of 40% were used in our experiments. Other apparatus used included tissue disintegrator, rotary evaporators, nitrogen concentrator, oscillator, water purification system, electronic analytic balance (0.01 mg), electronic analytic balance (1 mg), glass chromatography column (300 mm×18 mm), and vortex micromixer.

Procedure

A standard stock solution of AM (1000 mg/L)was prepared by dissolving 10 mg of the standard monomer in 10 mL of methanol solution, and stored at –20 ℃. A midst solution (100 mg/L) was prepared by diluting 1.00 mL of stock solution in 9 mL of methanol solution, and stored at –20 ℃. Solution I (10.0 mg/L) was prepared by diluting 1.00 mL of midst solution in 9 mL of 0.1% methanoic acid solution, for instant use. Solution Ⅱ (1.00 mg/L) was prepared by diluting 1.00 mL of solution I in 9 mL of 0.1% methanoic acid solution, for instant use.

A standard stock solution of $^{13}C_3$-AM I (1 000 mg/L)was prepared by dissolving 10 mg of $^{13}C_3$-AM standard monomer in 10 mL of methanol solution, and stored at -20℃. A standard stock solution of $^{13}C_3$-AM Ⅱ (10 mg/L)was prepared by dissolving 1.00 mL of $^{13}C_3$-AM standard solution I in 99 mL of methanol solution, and stored at –20 ℃. A $^{13}C_3$-AM solution (1.0 mg/L) was prepared by dissolving 1.00 mL of $^{13}C_3$-AM standard solution Ⅱ in 99 mL of 0.1% methanol solution, and stored at –20 ℃.

Two grams of the PAHG specimens was immersed in extra pure water (10 mL) with $^{13}C_3$- AM (10 μL) in the ratio of 10 mg/ L, oscillated for 30 min, and centrifuged for 10 min at 4 000 rpm. The supernatant liquor was kept.

The supernatant liquor and 8 g of ammonium sulfate were mixed, oscillated for 10 min and centrifuged for 10 min in 4 000 rpm. Ten grams of the supernatant liquor was kept for use, and 10 g of sodium sulfate anhydrous and 2 g of Extrelut™ 20 diatomite were put into a clean glass chromatography column. Then, 5 g of Extrelut™ 20 diatomite and the sample supernatant liquor were mixed and put into the chromatography column, eluted with 60 mL of hexane at a rate of 2 mL/min, and eluted again with 60 mL of ethyl acetate at a rate of 2 mL/min. The ethyl acetate eluate was collected, vaporized in a water bath of 45 ℃, deterged and vaporized 3 times with ethyl acetate (1 mL per time). Then the sample solution and 1 mL of 0.1% methanoic acid were mixed and oscillated. The super stratum organic phase was derived by the nitrogen flow. After 1mL of hexane was added, it was oscillated and centrifuged for 5 min at 3 500 rpm. The under layer water phase was filtered with a 0.22 μm filtration membrane and determined by HPLC-MS.

The sample solution and standard solutions were injected into HPLC-MS/MS system, and the peak area of AM and $^{13}C_3$-AM was recorded. The peak area ratio of AM (m/z55) and $^{13}C_3$-AM (m/z58) was calculated. The linear curve was protracted with the sample quantity (ng) of standard solutions and the peak area of AM (m/z55) and $^{13}C_3$-AM (m/z58).

The content of AM (X_n, μg/kg) in the samples was calculated by the following formula:

$$X_n = \frac{A_n \times m_s}{A_s \times RRF \times m}$$

where m_s indicates the $^{13}C_3$-AM content in the sample (ng), A_n the peak area of the objective, A_s the peak area of $^{13}C_3$-AM ration, m the sampling content (g).

The relative response factor (RRF) was calculated by the following formula:

$$RRF=\frac{An\times Cs}{As\times Cn}$$

where A_n is the peak area of the objective, Cn the content of the objective (ng/mL), A_s the peak area of $^{13}C_3$-AM ration, Cs the content of $^{13}C_3$-AM ration (ng/mL).

The accuracy was evaluated by recovery and the precision was evaluated by the relative standard deviation (RSD). Recovery was the ratio of the response (peak area) to the sample and standard solutions. RSD= standard deviation /mean×100%.

RESULTS

The linear equation is y=165.22x-2.2547. The correlation coefficient (R^2) is 0. 9983. The assay was linear between 0 and 3 000 μg/L and suitable for the determination of AM in medical PAHG (Fig. 1). After the extraction and purification of medical PAHG samples, the mass spectra were obtained by HPLC—MS/MS (Fig. 2). There was an interference peak before the retention time of AM with a distinct dividing line. The peak shape of the objective was symmetrical and sharp. The concentration of AM in PAHG ranged from 3.9×10^{-9} to 3.1×10^{-8} g/L ($14.39\pm6.40\times10^{-9}$g/L) in the 20 clinical cases. The outcomes were under the ISO quality standard and biological evaluation standards of national medical devices[5]. The RSD was calculated to be 6.20% using the integral data from the concentration ration of 50 μg/L. The recovery rate was 103.1%.

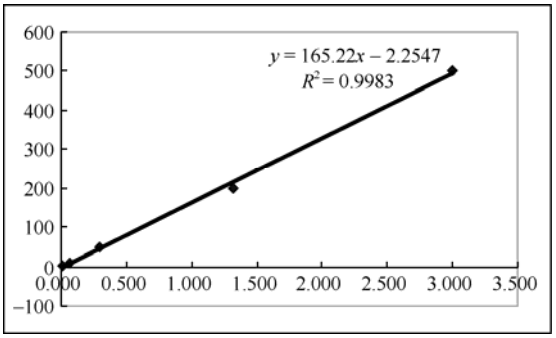

FIG. 1. AM calibration curve which is plotted by comparing the peak area with AM concentration. Correlation coefficient (R^2) is 0.9983 and linear equation is y= 165.22x-2.2547. The assay is linear between 0 and 3 000 μg/L.

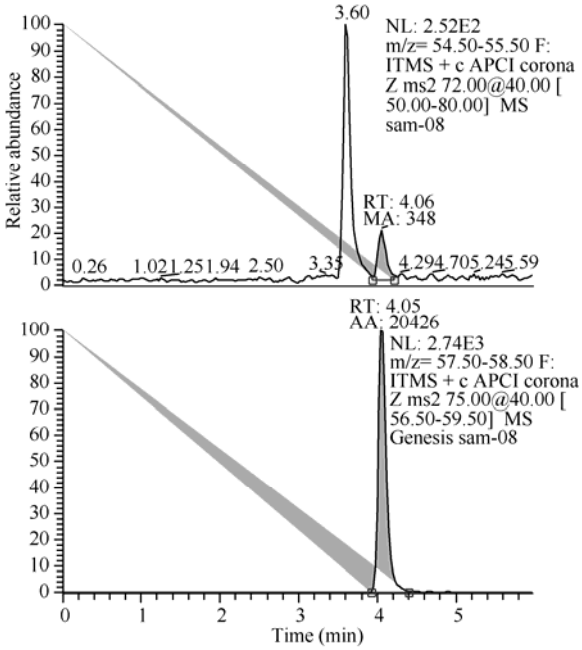

FIG. 2. Mass spectra of AM in PAHG (72→55) and internal label $^{13}C_3$ AM (75→58). There is an interference peak before the retention time of AM with a distinct dividing line.

DISCUSSION

Ultraviolet spectrophotometry, gas chromatography and liquid chromatography were used to determine AM, but the results were not satisfactory. These methods had high detecting limits and interference factors could significantly influence the results[6]. Consequently, AM could not have been accurately determined.

HPLC consists of a collocating high pressure pump and a high sensitive detector. Its speed is fast with high efficiency. Nearly all compounds including high polarity or ion substances and macromolecule substances can be determined by HPLC. Some researchers usually use HPLC to determine AM in food and water[7-9]. Although HPLC is simple and accurate with good reproducibility, it has some limitations in comparison with HPLC-MS.

HPLC-MS[10] is a system with a high performance separation and can be used for quantitative and qualitative determination of multiple compounds. It can also provide the compound with molecular weight and structure information[11-12]. HPLC-MS is able to analyze the liquid sample and is harmless to the analyzer[13]. The sample preparation of HPLC-MS/MS is much easier[14]. In July of 2002, WHO and FAO authorized GC–MS and LC-MS[15]. US Food and Drug Administration (FDA) announced

a draft of detection and quantification of AM in foods in February of 2003[16]. Consequently, HPLC-MS is a more convenient method for monitoring AM in medical PAHG.

The reason for the interference peak before the retention time of AM in all of the 20 PAHG samples is unknown. The interference peak has distinct dividing line. There is no interference peak in the detection of PAHG standard. Whether the sample is contaminated with blood or unknown decomposition of PAHG occurs in the human body is an enigma. There is no good sample preparation for the human blood serum[17]. The interference of blood components cannot be excluded. For patients with PAHG injection, surgical operation is the best way to get rid of the gel.

In nature, PAHG degrades slowly (by heat, shear, hydrolyzation, oxygenation, catalyzed oxygenation and biocatalysis) into oligomers, such as AM which damages the human body directly or indirectly. It cannot exclude the possibility that PAHG may degrade into alternative compounds, other than AM in the human body. The compounds may change the inner environments, such as pH, which can cause muscle ache and systemic complications. Once the decomposition occurs, damage to the human body is inevitable. The level of AM in water defined by WHO is 1 μg/L. Xi[5] has reported that AM is nontoxic if its concentration in PAHG is lower than 1.00×10^{-8} g/mL. In our study, the concentration of AM in PAHG is $14.39\pm6.40\times10^{-9}$g/L in the 20 clinical samples, indicating that AM in medical PAHG is within its normal range. It has been reported that residual AM in amazing gel is lower than 1.10×10^{-6} g/g[18] and 1.3×10^{-9} g/g after 6 months[19], which are consistent with our findings. Therefore, HPLC-MS is a more convenient method for monitoring AM in medical PAHG.

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