Original

Determination of (*R*)−3−chlorostyrene oxide in bulk chemicals Using Liquid Chromatography / Atmospheric Pressure Chemical Ionization Mass Spectrometry

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Received for review May 31, 2004. Revised manuscript received July 16, 2004. Accepted August 5, 2004.

Abstract

Liquid chromatography/mass spectrometry (LC/MS) operating condition and the sample preparation method were developed and fully validated according to the guidelines issued by Internal Conference on Harmonization (ICH) for the determination of (*R*)−3−chlorostyrene oxide (RCSO) present in bulk chemicals. RCSO is the raw material of a certain promising medical chemical. The chromatographic separation of RCSO and bulk chemical was achieved using a mixture of methanol and acetate buffer (3:2) as the mobile phase. Mass spectrometric analysis was performed by atmospheric pressure chemical ionization (APCI) mode with positive ion detection. Single ion monitoring (SIM) scan mode of m/z 172.1 was used to quantitatively determine RCSO. Analysis by LC/APCI−MS was carried out within 10 min. Limits of detection and quantitation level of RCSO in bulk chemicals were estimated to be 5 and 10 ng/mg chemical corresponding to 5 ppm and 10 ppm, respectively. Calibration curve of RCSO exhibited the linearity in the RCSO concentration of 10−120 ng/mg chemical (ppm). Accuracy and intermediate precision of this method were 0.4% and 2.8%, respectively. This analytical method as the impurity testing for quality control was successfully applied to bulk chemicals. RCSO was not detected at all in 32 batches of bulk chemicals tentatively manufactured in our laboratory.

Keywords: (R)−3−chlorostyrene oxide, validation, liquid chromatography−atmospheric pressure chemical ionization mass spectrometry, ICH guideline

1. Introduction

Styrene oxide is the important chiral stating material for the pharmaceutical industry. [1−4] Particularly, (*R*)−3−chlorostyrene oxide (RCSO) is the common key material for the preparation of antagonists of central and peripheral dopamine receptors [5], and several promising medicinal substances showing antiobesity and antidiabetic activities [6]. In the manufacture of a certain promising chemical in our laboratories, RCSO, that would be positive to the mutagenicity test, is used as the raw material. Chemical structure of RCSO is provided in Figure 1.

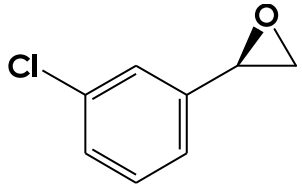

Figure 1. Chemical structure of RCSO.

Under the Internal Conference on Harmonization (ICH) guideline Q3A (R) “Impurities in the Drug Substances” [7], the organic impurities present in the drug substance should be lower than the qualification threshold of 0.05% or 0.15% depending upon maximum daily dose of the new drug substance. Qualification threshold is defined by the ratio of amounts of organic impurities and bulk drug substance (organic impurity / bulk drug substance).

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Especially, in the case of highly toxic substance, lower qualification threshold than 0.05% or 0.15% can be important. Whenever the content of organic impurities exceed the qualification threshold, the safety−study using animal must be carried out to clarify the margin of safety of impurity. Therefore, it is crucial to establish the quantitative determination method of RCSO contaminated in bulk chemicals. However, it is difficult to determine the RCSO level in bulk chemicals at ppm level by the conventional HPLC method due to poor absorption of RCSO molecule for UV−Vis detector equipped in the HPLC system. In our knowledge, the determination method of RCSO at ppm level has been published nowhere else. This report describes the validation characteristics of analytical method for determination of RCSO in bulk chemicals and application to quality control of bulk chemicals.

2. Experimental

2.1. Materials and reagents

Bulk chemicals were tentatively manufactured in Dainippon Pharmaceutical Co., Ltd. (Osaka, Japan). RCSO was obtained from Mitsubishi Rayon Co., Ltd. (Tokyo, Japan) and used as the starting material of the promising chemical. RCSO is clear and colorless liquid (boiling point: 120.16℃). Ammonium acetate and acetic acid of reagent grade, and acetonitrile, methanol and water of HPLC grade were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Reagents were used without further purification. Ammonium acetate buffer was prepared by dissolving 0.77 g of ammonium acetate in 800 mL of water, adjusting pH of 4.7 using diluted acetic acid, and adding water to make 1000 mL.

2.2. Instrumentation and conditions

The HPLC system was Hitachi (Tokyo, Japan) D−7000 system consisted of a Hitachi Model L−7100 pump, auto−sampler L−7200, column oven L−7300 equipped with cooling unit, UV−VIS detector L−7420 and an interface D−7000. Samples were hold at 10℃ to prevent the vaporization of RCSO from the sample solution. The LC/MS system was HP 1100 system (degasser: G 1322 A, pump: G 1312 A, auto−sampler: G 1367 A and column controller: G 1316 A) equipped with mass detector 1100 Series LC/MSD (Agilent, CA, USA) used in positive ion (PI) selected ion monitoring (SIM) mode at m/z 172.1. The voltages of fragmentor and capillary were set at 90 V and 2200 V, respectively. Neblizer pressure was set at 50 p.s.i., and drying gas at 4.0 mL/min. Drying gas temperature was 340℃, and vaporizer temperature 425℃. Injection volume was 50 μL. Analyses were carried out on a conventional ODS−column, Hydrosphere C 18 (75 mm × 4.6 mm i.d.) from YMC (Kyoto, Japan) with a mobile phase consisted of methanol and ammonium acetate buffer (3:2), unless otherwise specified. The mobile phase was isocratically pumped at 0.5 mL/min.

2.3. Preparation of the RCSO quality control solutions

Chemicals spiked with RCSO was dissolved in the mixture of acetonitrile and water (1:1). In this study, the concentration of chemical was adjusted to 1 mg/mL, unless otherwise stated. Concentrations of RCSO spiked onto bulk chemicals were adjusted to 3−1000 ng/mL, corresponding to 3−1000 ppm of RCSO contaminated in bulk chemical. These solutions were employed as the quality control solution to validate the analytical method according to the ICH guidelines, Q2A "Text on Validation of Analytical Procedures" [8] and Q2B "Validation of Analytical Procedures: Methodology" [9]. Calibration curve was drawn by plotting peak area of RCSO in the chromatogram versus the RCSO concentration injected into LC/MS. Accuracy (%) of the method was defined by the analysis of the quality control solutions prepared at three concentration levels for accuracy spanning the calibration range. Accuracy was evaluated according to the following equation:

$$Accuracy = \left(\frac{X - C}{C} \right) \times 100$$

where X is the determined RCSO concentration and C is the theoretical RCSO concentration in the quality control solution. Precision of the method was evaluated by analyzing a certain quality control solution (RCSO concentration of 100 ng/mg chemicals) successively for "repeatability", and on 6 different days for "intermediate precision". Precision was expressed as the percentage of the relative standard deviation (RSD: %) of replicate measurements.

2.4. Application to bulk chemicals

Bulk chemicals of 32 batches were dissolved in the mixture of acetonitrile and water (1:1) to be 1 mg/mL. This solution and the standard solution containing RCSO at 100 ng/mL were injected into LC/APCI−MS. The levels of RCSO contaminated in bulk chemicals were calculated by the comparison of peak areas of RCSO obtained from these solutions.

3. Results

3.1. Specificity

To demonstrate the specificity of this method, the solution containing both RCSO (1000 ng/mL) and chemical (1 mg/mL) was injected into conventional HPLC with UV−detector. The separation between RCSO and chemical is shown in Figure 2. RCSO and bulk chemicals were completely resolved within 10 minutes with resolution of 9.3. By this conventional HPLC method, limit of determination of RCSO was about 0.02% (RCSO / chemical). In the analytical procedure with LC/MS, the main peak (chemical) was diverted to waste prior to entering the mass spectrometer to avoid saturation of the MS detector.

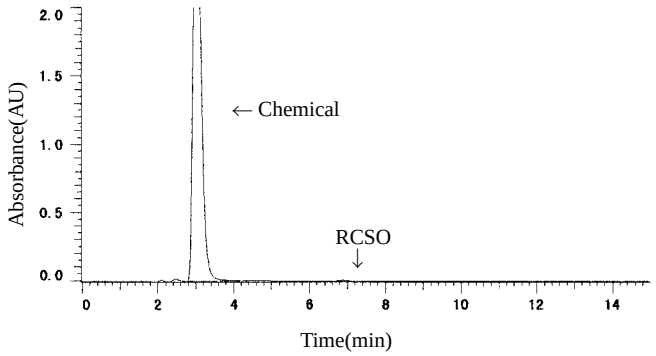

Figure 2. Separation of chemical and RCSO. HPLC operating conditions: column, Hydrosphere C 18 (75 mm in length and 4.6 mm in inner diameter); mobile phase, a mixture of methanol and ammonium acetate buffer (3: 2); flow rate, 0.5 mL/min; detection, UV at 219 nm.

3.2. Conditions for LC/MS

Before interfacing the LC separation to the MS system, flow injection analyses were conducted using the atmospheric pressure chemical ionization (APCI) and the electrospray ionization (ESI) sources in both positive and negative ion modes. The results from ESI ion source screening were insubstantial. No signal of RCSO was detected in either positive or negative ion mode. Detectable ion signals were realized during the APCI ion source screening in both positive and negative ion modes. A strong signal corresponding to m/z 172.1 ($[M + NH_4]^+$) was observed in the mass spectrum (Figure 3). After establishing that the positive ion APCI source was most suitable source for the determination of RCSO, other parameters such as fragmentor voltage, capillary voltage, nebulizer pressure, drying gas flow−rate, drying gas temperature, vaporizer temperature were optimized in order to obtain the intense signal of RCSO. Selected ion monitoring (SIM) scan mode was selected to determine RCSO.

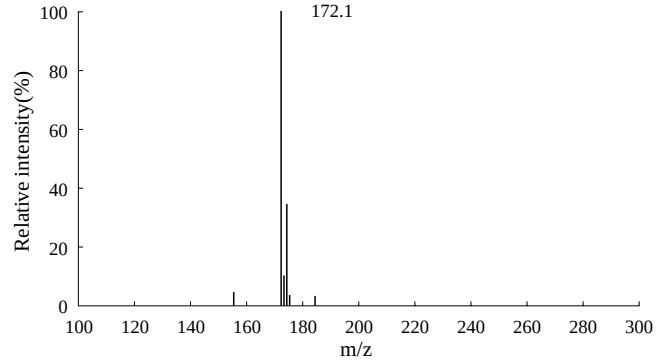

Figure 3. Mass spectrum of RCSO obtained by LC/APCI−MS.

3.3. Limits of detection and quantitation

The limits of detection (LOD) and quantitation (LOQ) were defined by measuring six times the signal of RCSO. The amount of RCSO (ng/mg chemical: ppm) injected into LC/APCI−MS and RSD of peak areas is presented in Table 1. The LOD and LOQ were taken as the concentration levels at which the RSD were less than 30% and 10%, respectively. The LOD and LOQ were 5 ppm and 10 ppm of the ratio of RCSO in bulk chemical.

Table 1. RCSO level injected into LC/APCI−MS and its relative standard deviation of peak area (n = 6)

RCSO level (ng/mg chemical: ppm)	Relative standard deviation (%)
3	56.1
5	26.3
10	6.6

3.4. Linearity

The linearity between the peak area and the amount of RCSO injected was evaluated in the range of LOQ (10 ng/mg chemical) to 120 ng/mg chemical. The plot is depicted in Figure 4. The calibration graph passed through the origin (as judged by the 95 % confidence level) with correlation coefficient of more than 0.999.

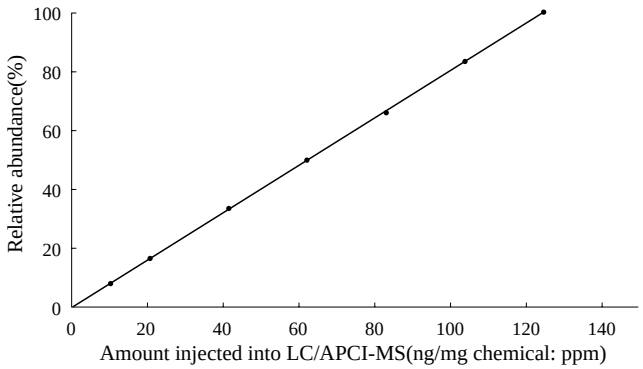

Figure 4. Calibration curve generated from peak area and the amount of RCSO injected into LC/APCI−MS. Relative abundance: 100% = 2.05 E 4.

3.5. Accuracy

Accuracy was verified by analyzing the given quality control solutions. Results are summarized in Table 2. Accuracy shown in Table 2 is excellent with values ranging between−4.9% and 3.8% for individual values, and 0.4% for total accuracy. Lower and upper limits of estimated recovery average are−6.2% and 7.0%, respectively, at the 95% confidence level, indicating this method is accurate enough as the impurity testing for the purpose of determination of RCSO in the bulk chemicals.

Table 2. Accuracy of the method

RCSO level (ng/mg chemical: ppm)	Individual accuracy (%)[1)]	Total accuracy (%)[2)]
10	−4.9	
100	2.4	0.4
120	3.8	

1) n = 3. 2) n = 9.

3.6. Precision

Precision was evaluated as the RSD of RCSO assayed values by six replicate analyses of the solution containing both RCSO (100 ng/mL) and chemical (1 mg/mL) by LC/APCI−MS. RCSO was successively injected into LC/APCI−MS six times. The RSD of RCSO assayed values (reproducibility) is 1.1%, and the upper 95 percent confidence limit was 2.9%. Two analysts, with three columns, analyzed solutions containing RCSO and chemical on the different days using three mobile phases separately prepared. Inter−mediate precision on six separate occasions was 2.8%, and upper limit of RSD was estimated to be 4.8% at 95% confidence level. These results indicate that this analytical method is precise enough as the impurity testing for the purpose of determination of RCSO in the bulk chemicals.

3.7. Application to bulk chemicals

Detection of RCSO in bulk chemicals manufactured in our laboratories was performed with 32 batches. The time span for the chromatographic run was 10 minutes. The result is summarized in Table 3 and typical chromatograms are shown in Figure 5. It turned out that RCSO was not detected in bulk chemicals tested at all.

Table 3. Detection of RCSO in bulk chemicals

Batch no.	RCSO level (ng/mg chemical: ppm)
A 1, A 2 B 1, B 2, B 3 C 1, C 2, C 3, C 4 D 1, D 2, D 3, D 4, D 5, D 6, D 7, D 8, D 9 E 1, E 2, E 3, E 4, E 5, E 6, E 7, E 8, E 9 F 1, F 2, F 3, F 4, F 5	< 5

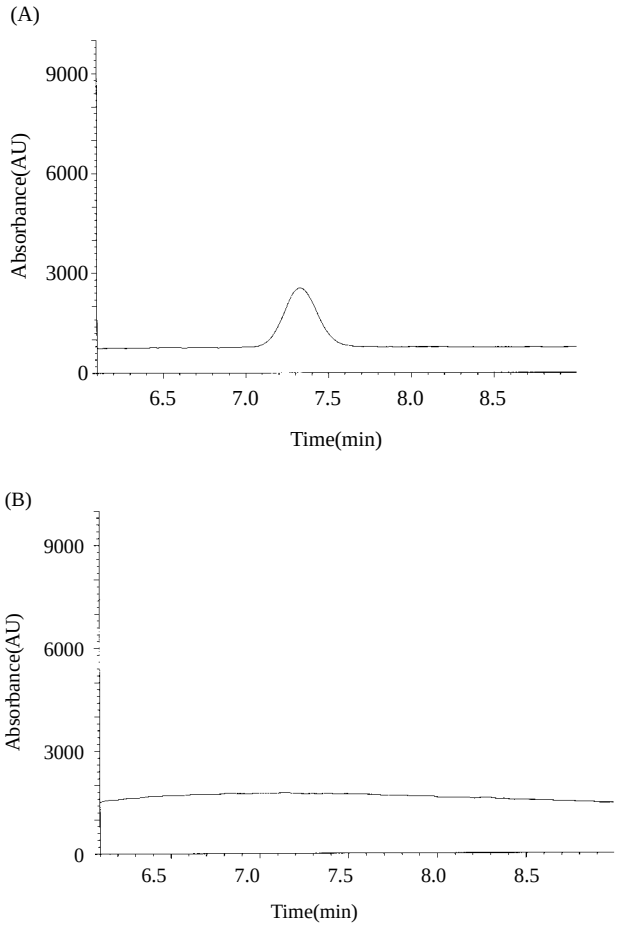

Figure 5. Selected ion chromatograms of the standard solution (A) and the sample solution prepared from batch #F 1 (B).

4. Conclusion

Testing method for determination of RCSO in bulk chemicals was developed. The RCSO and bulk chemical are completely resolved by liquid chromatography, and RCSO is determined by mass spectrometer in the APCI mode. Analytical procedure was completed within 10 minutes. This method exhibited a good accuracy and reproducibility, and low limits of detection (5 ppm) and quantitation (10 ppm). The described method was successfully applied to 32 batches of the bulk chemicals, resulted in no detection of RCSO. This analytical result indicate that it is unnecessary to conduct the safety study, which is time−consuming and costly, in order to clarify the toxicity of RCSO on the treated animals.

Acknowledgement

We thank Mr. Nishioka and Miss Umeki for many useful discussions on LC/MS. We are also grateful to Miss Shiono for her experimental help.

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