Research Article

Identification of the Related Substances in Ampicillin Capsule by Rapid Resolution Liquid Chromatography Coupled with Electrospray Ionization Tandem Mass Spectrometry

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Rapid Resolution Liquid Chromatography coupled with Electrospray Ionization Tandem Mass Spectrometry (RRLC-ESI-MSn) was used to separate and identify related substances in ampicillin capsule. The fragmentation behaviors of related substances were used to identify their chemical structures. Finally, a total of 13 related substances in ampicillin capsule were identified, including four identified components for the first time and three groups of isomers on the basis of the exact mass, fragmentation behaviors, retention time, and chemical structures in the literature. This study avoided time-consuming and complex chemosynthesis of related substances of ampicillin and the results could be useful for the quality control of ampicillin capsule to guarantee its safety in clinic. In the meantime, it provided a good example for the rapid identification of chemical structures of related substances of drugs.

1. Introduction

Ampicillin is an important semisynthetic β-lactam antibiotic and it is still widely used nowadays because of its good efficacy in urinary tract infections, respiratory infections, and other diseases caused by germs and bacteria. In recent years, the requirement of quality control for related substances in chemicals became stricter no matter in structure confirmation or content limitation. Ampicillin was especially degradable in presence of aqueous solution or humid storage environment, which would lead to the formation of a variety of degradation products [1]. These related substances (the related substances previously reported were shown in Table 1) would have a great influence on the quality of the products and clinical medication safety.

Although there has been much research on the related substances of ampicillin [2, 3], it is not completely explicit so far. To ensure the clinical safety and meet the new requirement of related substances in chemicals [4], it is still necessary to conduct further studies to develop a rapid and efficient method to describe in more detail the related substances of ampicillin capsule.

Many analytical methods including high-performance liquid chromatography (HPLC) [1, 5], high-performance capillary electrophoresis (HPCE) [6], high-performance liquid chromatography-atmospheric pressure chemical ionization mass (HPLC-APCI-MS) [7], and high-performance liquid chromatography-electrospray mass spectrometry (HPLC-ESI-MS) [8, 9] had been utilized for the analysis of ampicillin. Among these methods, LC-ESI-MS had been shown to be a powerful technique for the analysis of ampicillin and its related substances due to its excellent ability in separation and identification.

In this paper, a simple, rapid, and sensitive Rapid Resolution Liquid Chromatography coupled with Electrospray Ionization Tandem Mass Spectrometry (RRLC-ESI-MSn) method was established for the identification of the related substances in ampicillin capsule. The result suggested that this technique...
Table 1: The structures of the known related substances of ampicillin.

| Number | Name of related substances                  | Chemical structure                                      |
|--------|---------------------------------------------|---------------------------------------------------------|
| 1      | 6-Aminopenicillanic acid (6-APA)            | ![Chemical structure](image1)                           |
| 2      | L-Ampicillin                                | ![Chemical structure](image2)                           |
| 3      | Diketopiperazines of ampicillin             | ![Chemical structure](image3)                           |
| 4      | Ampicilloic acid                            | ![Chemical structure](image4)                           |
| 5      | Ampilloic acid                              | ![Chemical structure](image5)                           |
| 6      | Ampicillinyl-D-phenylglycine                | ![Chemical structure](image6)                           |
| Number | Name of related substances                  | Chemical structure |
|--------|--------------------------------------------|--------------------|
| 7      | (3R,6R)-3,6-Diphenylpiperazine-2,5-dione    | ![Chemical Structure](image1) |
| 8      | 3-Phenylpyrazin-2-ol                       | ![Chemical Structure](image2) |
| 9      | D-Phenylglycylampicillin                   | ![Chemical Structure](image3) |
| 10     | N-Pivaloyl-6-APA                           | ![Chemical Structure](image4) |
| 11     | N-Pivaloylphenylglycine                    | ![Chemical Structure](image5) |
| 12     | D-Phenylglycine                            | ![Chemical Structure](image6) |
| Number | Name of related substances | Chemical structure |
|--------|----------------------------|--------------------|
| 13     | Open-cycle dimer           | ![Chemical structure for open-cycle dimer 13](image) |
| 14     | Closed-cycle dimer         | ![Chemical structure for closed-cycle dimer 14](image) |
| 15     | Open-cycle trimer          | ![Chemical structure for open-cycle trimer 15](image) |
| 16     | Closed-cycle trimer        | ![Chemical structure for closed-cycle trimer 16](image) |
| 17     | Open-cycle tetramer \( (n = 2) \) | ![Chemical structure for open-cycle tetramer 17](image) |
could facilitate rapid and accurate identification of related substances in ampicillin capsule.

2. Experimental

2.1. Chemicals and Materials. Methanol (HPLC grade) was purchased from Fisher Scientific (Pittsburgh, PA, USA). Formic acid (HPLC grade) was obtained from Acros Organics (Geel, Belgium). Deionized water was further purified with a Milli-Q water system (Bedford, Massachusetts, USA). Ampicillin capsule was purchased from DAVA Pharmaceuticals, Inc. (Huntville, AL, USA).

The chromatographic separation was performed with an Agilent 1200 series Rapid Resolution Liquid Chromatography system (Agilent Technologies, USA), equipped with a binary pump, a microvacuum degasser, a high-performance autosampler, a column compartment, a diode array detector, and a MS detector. The samples were separated on a 1.8 μm Agilent Zorbax XDB-C<sub>18</sub> column (50 mm × 4.6 mm) at a flow rate of 0.4 mL min<sup>-1</sup>. The mobile phases consisted of 0.1% formic acid solution (A) and methanol (B). The optimized RRLC elution conditions were as follows: 0–2 min, 10% B; 2–10 min, 10–20% B; 10–20 min, 20–50% B; 20–25 min, 50% B; 25–25.1 min, 50–10% B; 25.1–30 min, 10% B. DAD spectra were acquired over a scan range of 190–400 nm. The sample volume injected was 1 μL. Agilent 6320 mass spectrometer with an Agilent ChemStation to control and process the data was performed with the ESI source in positive ion mode. The vaporizer temperature was maintained at 300 °C. The temperature of the drying gas was set at 350 °C. The flow rate of the drying gas and the pressure of the nebulizing gas were set at 12 L min<sup>-1</sup> and 35 psi, respectively. The capillary voltage was kept at 3.5 × 10<sup>4</sup> V. The mass spectrometer scanned from a mass-to-charge ratio (m/z) 100–900.

2.2. Preparation of Sample. The contents of ampicillin capsule (equivalent to 10 mg Ampicillin) were dissolved in 10 mL methanol and then filtered through a 0.22 μm syringe filter. An aliquot (1 μL) of the filtrate was subjected to RRLC-ESI-MS<sup>8</sup> for analysis.

![Figure 1: The total ion chromatography of ampicillin capsule (DAVA). The peaks were numbered according to their retention time.](image-url)

3. Results and Discussion

3.1. Investigation of the Fragmentation Patterns of Ampicillin. It was necessary to study the characterization of the mass spectra of the parent drug to identify the molecular structure of the related substances in ampicillin capsule. Identifications were based on the fact that the related substances of ampicillin usually contain structural fragments and analogous cleavage characteristic of the parent drug. Structural information and fragmentation mechanisms had been deduced from ions in the mass and collision spectra. This knowledge was useful in the analysis and identification of related substances in ampicillin capsule. We utilized knowledge of characteristic fragment ions of ampicillin and its related substances to identify their structures. Figures 1 and 2 showed the detailed total ion chromatography (TIC) and mass spectrum of ampicillin and its related substances, respectively.

Ampicillin yielded an abundant ion in the ESI mass spectrum at m/z 350.1. The ESI mass spectrum of this ion (m/z 350.1) was shown in Figure 2. The fragment ions at m/z 106.2 and 160.0 were reported to arise from the benzylamine group and the thiazolidine ring. The fragment ion at m/z 192.0 was
Figure 2: The mass spectrum of 16 chemicals in ampicillin capsule.
proposed to arise as a result of losing a $-\text{NH}_2$ group at the benzylamine side chain followed by an oxygen rearrangement and cleavage of the $\beta$-lactam ring. The fragment ion at $m/z$ 174.0 could be attributed to the loss of $\text{H}_2\text{O}$ from the fragment at $m/z$ 192.0, but it might arise from other pathways. The proposed fragmentation pathways of ampicillin were shown in Figure 3.

3.2. Identification of the Known Related Substances in Ampicillin Capsule. This part of the investigation focused on the characterization of the ESI-MS properties of the parent drug and its known related substances. Table 2 showed the chromatographic and mass spectral characteristics of the detected related substances in ampicillin capsule.

Peak 1 and Peak 2 showed the same MS data. All of them produced protonated quasimolecular ion at $m/z$ 368.1 $[\text{M + H}]^+$, major ions at $m/z$ 324.1, 307.1, 279.2, and 175.1 in ESI$^+$ mode. Based on diagnostic ions ($m/z$ 324.1, 307.1, and 175.1) and comparison with the published literature of known related substances of ampicillin [10], Peak 1 and Peak 2 were identified as isomers of ampicilloic acid. (5S,6R) or (5R,6R) ampicilloic acids were the two groups of ampicilloic
acid isomers which were reported to be the metabolites and degradation products of ampicillin [1]. According to the retention behavior in reversed-phase chromatography of Peak 1 and Peak 2 and the related literature [1], Peak 1 and Peak 2 were tentatively identified as (5S, 6R) ampicilloic acid and (5R, 6R) ampicilloic acid, respectively. Figure 4 showed the proposed MS fragmentation pathway for the fragmentation ions of ampicilloic acid.

Peak 3 produced a protonated molecular ion at m/z 350.1 [M + H]^+, fragment ions at m/z 333.0 [M – NH3]^+, 192.0, 174.0, 160.0, and 106.1 in ESI^+ mode. Peak 5 was clearly identified as ampicillin based on comparison of its retention time and mass spectrometric data with reference standards [8]. Peak 3 showed the same fragment ions, fragmentation pattern, and characteristic ions as Peak 5. Therefore, we could conclude that Peak 3 was an isomer of Peak 5. Considering that Peak 3 had a much shorter retention time than Peak 5, and with the comparison of related substances reported in the literature [1], Peak 3 was tentatively identified as L-ampicillin. Figure 5 showed the proposed MS fragmentation pathway for the fragmentation ions of L-ampicillin.

Peak 4 gave a protonated molecular ion [M + H]^+ with an m/z value of 324.1, major fragment ions at m/z 307.0, 279.1, 201.0, 175.0, 147.1, 128.1, and 106.1 in ESI^+ mode. Peak 4 was tentatively identified as (5R) or (5S) ampioiloic acid based on its characteristic ions (m/z 324.1, 307.0, 279.1, 128.1, and

![Figure 4: Proposed fragmentation pathway for the fragmentation ions of ampicilloic acid (m/z 368).](image)

**Table 2: Results of identification of the known related substances in ampicillin capsule.**

| Peak number | RT (min) | MS (m/z)       | MS^2 (m/z)     | Identification                  |
|-------------|----------|----------------|----------------|----------------------------------|
| 1           | 1.8      | 368.1 [M + H]^+| 324.1, 307.1, 279.2 | (5S, 6R) ampicilloic acid       |
| 2           | 2.2      | 368.1 [M + H]^+| 324.1, 307.2, 279.2, 175.1 | (5R, 6R) ampicilloic acid       |
| 3           | 2.5      | 350.1 [M + H]^+| 333.0, 192.0, 174.0, 160.0, 106.1 | L-Ampicillin                   |
| 4           | 2.8      | 324.1 [M + H]^+| 307.0, 279.1, 201.0, 175.0, 147.1, 128.1, 106.1 | (5S) or (5R) ampioiloic acids  |
| 5           | 4.2      | 350.1 [M + H]^+| 333.1, 192.0, 174.0, 159.9, 106.2 | Ampicillin                     |
| 6           | 8.1      | 364.1 [M + K^+H]^+| 191.9, 174.0, 128.0, 106.2 | (5S) or (5R) ampioiloic acids  |
| 7           | 9.0      | 382.1 [M + MeOH]^+| 331.1, 223.0, 206.0, 160.0, 106.2 | Diketopiperazines of ampicillin|
| 10          | 12.3     | 483.1 [M + H]^+| 439.1, 350.0, 267.0, 239.1 | D-Phenylglycylampicillin       |
| 15          | 17.0     | 699.1 [M + H]^+| 540.1, 381.1, 248.0 | Closed-cycle dimer              |
| 16          | 19.3     | 524.8 [1/2M + H]^+| 889.3, 730.3, 571.2, 160.0 | Closed-cycle trimer             |
106.1) and comparison with the published literature of known related substances of ampicillin [10]. Peak 6 produced major fragment ions at m/z 364.1 \([\text{M + K + H}]^+\), 191.9, 174.0, 128.0, and 106.2. They all had similar MS fragmentation patterns \((m/z \ 191.9, \ 174.0, \ 128.0, \ \text{and} \ 106.2)\). By comparison with the published literature [1], Peak 6 was tentatively identified as (5R) or (5S) ampioiloic acid. (5S) or (5R) ampioiloic acids were the isomers of ampioiloic acids which were reported to be metabolites or degradation products of ampicillin [1]. However, the exact structure of these two components could not be determined due to the limited information. Figure 6 showed the proposed MS fragmentation pathway for the fragmentation ions of ampioiloic acids.

Peak 7 had a molecular weight of 350 \([\text{M + MeOH + H}]^+\), m/z 382.1) and five major fragment ions were observed at m/z 331.1, 223.0 \([191 + \text{MeOH}]^+\), 206.0 \([174 + \text{MeOH}]^+\), 160.0, and 106.2. As it was reported [8], the two characteristic fragment ions m/z 160.0 and 106.2 were the representative fragment ions of ampicillin. By comparison with the published literature [7], this component was tentatively identified.
as diketopiperazines of ampicillin. Figure 7 showed the proposed MS fragmentation pathway for the fragmentation ion of diketopiperazines of ampicillin.

Peak 10 produced a protonated molecular ion at $m/z$ 483.1 $[M + H]^+$, the major fragment ions at $m/z$ 439.1, 350.0, 267.0, and 239.1 in ESI$^+$ mode. Fragment ion at $m/z$ 439.1 could be attributed to loss of one $–$COOH from the ion ($m/z$ 483.1). Based on the mass spectra, Peak 10 was identified as D-phenylglycylampicillin [1, II]. Figure 8 showed the proposed MS fragmentation pathway for the fragmentation ion of D-phenylglycylampicillin.

Peak 15 had a molecular weight of $m/z$ 699.1 $[M + H]^+$ and three major fragment ions $m/z$ 540.1, 381.1, and 248.0 in ESI$^+$ mode. Upon collision-induced dissociation (CID),
the ion at 799.1 eliminated one molecule of thiazolidine ring to produce 799.1. The 540.1 ion could further lose one molecule of thiazolidine ring successively to give significant 381.1 fragment ion. Thus, Peak 15 was identified as closed-cycle dimer based on the published literature [1]. Closed-cycle dimer was the main cause of allergy, so that we must control the amount of this related substance in ampicillin capsule.

Peak 16 produced major fragment molecular ions at 524.8 [M + H]^+, 889.3, 730.3, 571.2, and 160.0 in ESI^+ mode. Upon CID, the ion at 1048 [M]^+ eliminated one molecule of thiazolidine ring to produce 889.3. The 889.3 ion could lose one molecule of thiazolidine ring successively to give significant 730.3 ion. The 730.3 ion could further lose one molecule of thiazolidine ring successively to give significant 571.2. The fragment ion 160.0 is characteristic thiazolidine ring of ampicillin. Thus, Peak 16 was identified as closed-cycle trimer based on the published literature [1]. Closed-cycle trimer was also the main cause of allergy, so that we must control the amount of this related substance in ampicillin capsule.

3.3. Identification of the Unknown Related Substances in Ampicillin Capsule. This part of the investigation was to identify the chemical structures of unknown related substances which were not yet reported in ampicillin capsule based on the mass fragment characterization and cleavage pathways of ampicillin and its known related substances. By means of the RRLC-ESI-MS^n experiments, in this part, chemical structures of four related substances were tentatively identified in ampicillin capsule for the first time. Table 3 showed the chromatographic and mass spectral characteristics of the above unknown related substances detected by RRLC-ESI-MS^n in ampicillin capsule.

| Peak number | RT (min) | MS (m/z) | MS^2 (m/z) | Identification |
|-------------|---------|----------|-------------|----------------|
| 9           | 11.1    | 566.1 [M + H]^+ | 407.1; 248.1; 191.0 | Ampicilloic acid and 6-APA oligomer |
| 12          | 13.9    | 548.1 [M + H]^+ | 443.1; 358.1; 199.0 | 6-APA ampicillin amide |
| 13          | 15.2    | 673.2 [M + H]^+ | 655.1; 514.1; 324.1; 191.0 | Ampilloic acids and ampicilloic acids oligomer |
| 14          | 15.5    | 483.1 [M + H]^+ | 439.1; 350.1; 239.1; 160.0 | Isomer of D-phenylglycyl ampicillin |

Figure 8: Proposed fragmentation pathway for the fragmentation ion of D-phenylglycylampicillin.
Peak 9 had a molecular weight of \textit{m/z} 566.1 [M + H]$^+$ and three major fragment ions \textit{m/z} 407.1, 248.1, and 191.0 in ESI$^+$ mode. The fragment ions at \textit{m/z} 407.1 and 248.1 eliminated one molecule of thiazolidine ring successively from ion at \textit{m/z} 566.1 [M + 1]$^+$. The fragment ion at \textit{m/z} 191.0 probably should be a characteristic fragment ion of ampicillin piperazine-2,5-dione [12]. Thus, Peak 9 was identified tentatively as ampicilloic acid and 6-aminopenicillanic acid (6-APA) oligomer. Figure 9 showed the proposed MS fragmentation pathway for the fragmentation ion of ampicilloic acid and 6-APA oligomer.

Peak 12 produced a protonated molecular ion at \textit{m/z} 548.1 [M + H]$^+$ and three major fragment ions at 443.1, 358.1, and 199.0. Based on fragment ions, Peak 12 was tentatively identified as 6-APA ampicillin amide. Figure 10 showed the proposed MS fragmentation pathway for the fragmentation ion of 6-APA ampicillin amide.

Peak 13 had a molecular weight of 673.2 [M + H]$^+$ and four major fragment ions \textit{m/z} 655.1, 514.1, 324.1, and 191.0. The fragment ions at \textit{m/z} 655.1 and 514.1 were attributed to the loss of one water molecule (18 Da) and one molecule of thiazolidine ring from ion at \textit{m/z} 673.2. The fragment ion at \textit{m/z} 324.1 was molecular weight of ampilloic acids. The fragment ion at \textit{m/z} 191.0 was the fragment ion of ampilloic acids. Peak 13 was identified tentatively as ampilloic acids and ampilloic acids oligomer. Figure II showed the proposed MS fragmentation pathway for the fragmentation ion of ampilloic acids and ampilloic acids oligomer.

Peak 14 produced a protonated molecular ion at \textit{m/z} 483.1 [M + H]$^+$, which was identified as the other isomer of
D-phenylglycylampicillin because Peak 14 and Peak 10 both showed the same diagnostic ions at \( m/z \) 439.1, 350.1, 239.1, and 160.0.

4. Conclusion

The RRLC-ESI-MS\(^n\) technique was successfully established to rapidly determine and identify the structures of the related substances in ampicillin capsule. RRLC is efficient in separating chemical compounds in a mixture, and MS provides abundant information for structural elucidation of the compounds when tandem mass spectrometry is applied [13]. Although ampioic acids, ampicilloic acid, and closed-cycle dimer had been investigated previously by LC-MS method, MS information and characteristic diagnostic ions of a number of components in ampicillin capsule were described simultaneously for the first time. None of the previously reported methods have led to so much chemical information on the related substances in ampicillin capsule. The results of this study had identified 13 out of 15 related substances in ampicillin capsule. Unfortunately, three groups of isomers (Peak 1 and Peak 2, Peak 4 and Peak 6, and Peak 10 and Peak 14) and condensation of amino and carboxyl groups (Peak 9, Peak 12, and Peak 13) could not be identified fully by current RRLC-ESI-MS\(^n\) information. Peak 8 and Peak 11 had not yet been identified based on current mass spectra information. In summary, this investigation had provided an example of the rapid identification of related substances in ampicillin capsule. The meaningful information for the related substances in ampicillin capsule could lead to the development of the understanding of the quality and safety of the drug.
Conflict of Interests

The authors declare that there is no conflict of interests.

Authors’ Contribution

Lei Zhang and Xian Long Cheng contributed equally in this work.

References

[1] Y. Zhu, E. Roets, Z. Ni, M. L. Moreno, E. Porqueras, and J. Hoogmartens, "Evaluation of liquid chromatography methods for the separation of ampicillin and its related substances," Journal of Pharmaceutical and Biomedical Analysis, vol. 14, no. 5, pp. 631–639, 1996.

[2] M. Shamsipur, Z. Talebpour, H. R. Bijanzadeh, and S. Tabatabaei, "Monitoring of ampicillin and its related substances by NMR," Journal of Pharmaceutical and Biomedical Analysis, vol. 30, no. 4, pp. 1075–1085, 2002.

[3] O. Shakoor and R. B. Taylor, "Analysis of ampicillin, cloxacillin and their related substances in capsules, syrups and suspensions by high-performance liquid chromatography," Analyst, vol. 121, no. 10, pp. 1473–1477, 1996.

[4] EMA, "Guideline on setting specifications for related impurities in antibiotics," http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/07/WC500129997.pdf.

[5] C. Larsen and H. Bundgaard, "Polymerization of penicillins. V. Separation, identification and quantitative determination of antigenic polymerization products in ampicillin sodium preparations by high performance liquid chromatography," Journal of Chromatography, vol. 147, pp. 143–150, 1978.

[6] C. Q. Niu and S. Q. Zhu, "Separation and determination of ampicillin polymers by high performance capillary electrophoresis," Acta Pharmaceutica Sinica, vol. 32, no. 3, pp. 207–209, 1997.
[7] S. Horimoto, T. Mayumi, K. Aoe, N. Nishimura, and T. Sato, "Analysis of β-lactam antibiotics by high performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry using bromoform," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 30, no. 4, pp. 1093–1102, 2002.

[8] R. F. Straub and R. D. Voyksner, "Determination of penicillin G, ampicillin, amoxicillin, cloxacillin and cephalirin by high-performance liquid chromatography—electrospray mass spectrometry," *Journal of Chromatography*, vol. 647, no. 1, pp. 167–181, 1993.

[9] E. Verdon, R. Fuselier, D. Hurtaud-Pessel, P. Couëdor, N. Cadieu, and M. Laurentie, "Stability of penicillin antibiotic residues in meat during storage ampicillin," *Journal of Chromatography A*, vol. 882, no. 1-2, pp. 135–143, 2000.

[10] S. Suwanrumpha and R. B. Freas, "Identification of metabolites of ampicillin using liquid chromatography/thermospray mass spectrometry and fast atom bombardment tandem mass spectrometry," *Biomedical and Environmental Mass Spectrometry*, vol. 18, no. 11, pp. 983–994, 1989.

[11] B. P. Commission and G. Britain, *British Pharmacopoeia 2011*, Stationery Office, 2010.

[12] S. Suwanrumpha, D. A. Flory, R. B. Freas, and M. L. Vestal, "Tandem mass spectrometric studies of the fragmentation of penicillins and their metabolites," *Biomedical and Environmental Mass Spectrometry*, vol. 16, no. 1-12, pp. 381–386, 1988.

[13] M. Ye, J. Han, H. Chen, J. Zheng, and D. Guo, "Analysis of phenolic compounds in rhubarbs using liquid chromatography coupled with electrospray ionization mass spectrometry," *Journal of the American Society for Mass Spectrometry*, vol. 18, no. 1, pp. 82–91, 2007.