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# Simultaneous determination of spironolactone and its active metabolite canrenone in human plasma by HPLC-APCI-MS

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**A sensitive and specific liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry (LC-APCI-MS) method for the simultaneous determination of spironolactone and its active metabolite canrenone in human plasma has been developed and validated. After the addition of estazolam as the internal standard (IS), plasma samples were extracted with methylene chloride : ethyl acetate mixture (20 : 80, v/v) and separated by high-performance liquid chromatography (HPLC) on a reversed-phase $C_{18}$ column with a mobile phase of methanol–water (57 : 43, v/v). Analytes were determined in a single quadrupole mass spectrometer using an atmospheric pressure chemical ionization (APCI) source. LC-APCI-MS was performed in the selected-ion monitoring (SIM) mode using target ions at *m/z* 341.25 for spironolactone and canrenone, *m/z* 295.05 for estazolam. The method was proved to be sensitive and specific by testing six different plasma batches. Calibration curves of spironolactone and canrenone were linear over the range 2–300 ng/ml. The within- and between-batch precisions (relative standard deviation (RSD)%) were lower than 10% and the accuracy ranged from 85 to 115%. The lower limit of quantification (LLOQ) was identifiable and reproducible at 2 ng/ml. The proposed method was successfully applied to study the pharmacokinetics of spironolactone and its major metabolite in healthy male Chinese volunteers.** 

**KEYWORDS:** spironolactone; canrenone; LC-APCI-MS; human plasma; pharmacokinetics

## INTRODUCTION

Spironolactone, (7$\alpha$,17$\alpha$)-7-(acetylthio)-17-hydroxy-3-oxo-pregn-4-ene-21-carboxylic acid $\gamma$-lactone (SL, see Fig. 1), is a competitive antagonist of aldosterone, which blocks the aldosterone binding to the renal receptor, resulting in sodium loss and potassium retention. It is widely used in the treatment of primary aldosteronism, essential hypertension, congestive cardiac failure and various edematous states.[1–3] After oral administration, spironolactone is extensively metabolized to a large number of compounds that can be divided into two classes.[4,5] In the first group, the sulfur atom of the parent drug is retained in the molecule. The second group is formed by the dethioacetylation of spironolactone and subsequent biotransformation steps. The most important compound in this second class is canrenone, (17$\alpha$)-17-hydroxy-3-oxopregna-4,6-diene-21-carboxylic acid $\gamma$-lactone (Fig. 1). It is pharmacologically active and accounts for the major part of the activity of spironolactone, at least after multiple oral administrations.[6]

In most previous investigations, the levels of canrenone in plasma were determined by a fluorimetric assay.[7,8] With the introduction of high-performance liquid chromatography (HPLC) methods to measure canrenone concentration, it became clear that the fluorimetric method was not specific for canrenone but measured other fluorescigenic metabolites as well. Afterward, Overdiek *et al.*[9,10] developed an HPLC method with an ultraviolet detector to determine spironolactone and its metabolites in human and animal serums. In these studies, only one wavelength (240 nm) was used, although the UV absorption maximum for canrenone appears at 280 nm. The limits of quantitation (LOQ), as derived from their calibration curves, were 12.5 ng/ml in guinea pig plasma[10] and 50 ng/ml in human plasma.[9] Varin *et al.*[11] determined spironolactone and its metabolites in human biological fluids by HPLC based on two sequential solid-phase extractions, which is an expensive and time-consuming method. Then, Jankowski *et al.*[12] modified the HPLC method, which used a one-step liquid–liquid extraction and a programmed switchover of the UV wavelength. However, the LOQ for spironolactone and its metabolites were only 10 ng/ml, which were not sensitive enough for pharmacokinetic research of spironolactone at clinical doses.

As an effort to reduce the time required for drug testing in biological fluids, our laboratory is continually investigating new strategies for improving sample preparation, chromatography and mass spectrometric detection. The aim of

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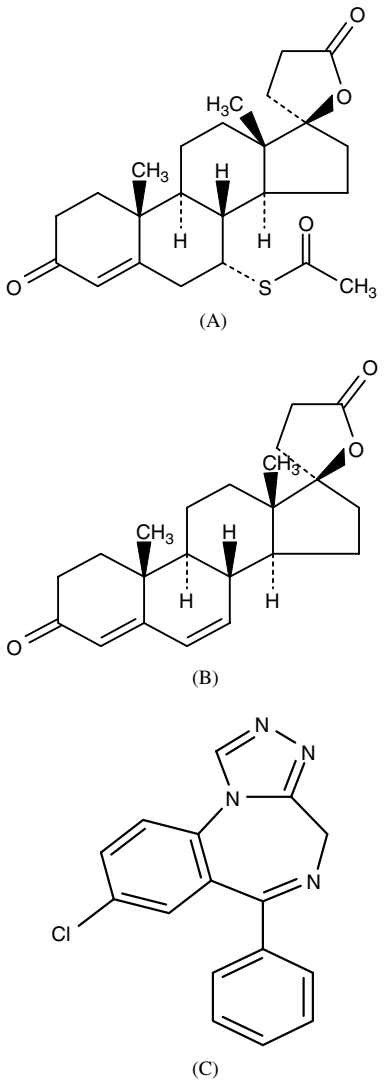


**Figure 1.** Chemical structure of spironolactone (A), canrenone (B) and estazolam (C).

the present study was to combine a fast HPLC technique with mass spectrometry in order to validate a robust and reproducible reversed-phase LC-MS method for the simultaneous determination of SL and its active metabolite canrenone in human plasma and to increase dramatically the sample throughput. Our interest was to establish a simple, single-step extraction technique and, at the same time, to employ the single quadrupole MS system. This method was to be validated to ensure proper quantification of SL and canrenone in human plasma down to the concentration limit of 2 ng/ml. At the same time, it was expected that the method would be efficient in analyzing a large number of plasma samples supporting pharmacokinetic, bioavailability or bioequivalence studies after administering therapeutic doses of spironolactone.

## EXPERIMENTAL

### Chemicals and reagents

Spironolactone test tablets (Batch No: 20040310), spironolactone reference standard (98.5% purity) and canrenone reference standard (91.4% purity) were identified and supplied by Jiangsu Fangqiang Pharmaceutical Factory (Jiangsu, P. R. China). Spironolactone reference tablets were purchased from Jiangsu Huanghai Pharmaceutical CO., LTD (Jiangsu, P. R. China, Batch No: 040728). Estazolam reference standard (99.3% purity, Batch No: 20011003M) was supplied by Hubei Pharmaceutical Factory (Hubei, P. R. China). Methanol was of chromatographically pure grade and purchased from Merck (Merck Company, Germany). Other chemicals were all of analytical grade. Deionized water was distilled before using. Other reagents were used as received.

### Instrumentation and operating conditions

*Liquid chromatographic conditions*

Liquid chromatography was performed using a Shimadzu LC-10AD HPLC system consisting of an autosampler (SIL-HTc). Chromatographic separation was carried out with an Agilent zorbax SB-$C_{18}$ (3.5 μm, 100 × 3.0 mm i.d.) column at 35 °C. The mobile phase consisted of methanol–water (57 : 43, v/v) and was set at a flow rate of 0.4 ml/min.

*Mass spectrometric conditions*

Mass spectrometric detection was performed on a Shimadzu LCMS-2010A quadrupole mass spectrometer equipped with an atmospheric pressure chemical ionization (APCI) interface. The APCI source was set at the positive ionization mode. The ions $[M - SCOCH_3]^+$, $m/z$ 341.25 for spironolactone; $[M + H]^+$, $m/z$ 341.25 for canrenone and $[M + H]^+$, $m/z$ 295.05 for estazolam were selected as detecting ions. The MS operating conditions were optimized as follows: drying gas, 2.5 l/min; APCI temperature, 300 °C; CDL temperature, 250 °C; block temperature, 200 °C; probe voltage, +4.5 kV. The quantification was performed via peak area ratio. Data acquisition and processing were accomplished using the Shimadzu LCMS solution software 2.04 Sul-H3 for LCMS-2010A system.

### Preparation of stock solutions

Stock solutions of spironolactone, canrenone and estazolam (internal standard (IS)) were prepared after correcting for purity.

The stock solution of spironolactone was prepared at 1.0 mg/ml in acetonitrile and was stored at 4 °C. Stock solutions of canrenone and estazolam (IS) were prepared in the mobile phase at a concentration of 1.0 mg/ml and were stored at 4 °C.

Working solutions of spironolactone and canrenone were prepared daily in the mobile phase by appropriate dilution of the stock solution at 0.1, 1.0 and 10.0 μg/ml.

The stock solution of estazolam was further diluted with the mobile phase to prepare the working IS solution containing 1.0 μg/ml of estazolam.

### Calibration curves

Calibration curves were prepared by spiking different samples of 1.0 ml blank plasma each with an appropriate volume of one of the above-mentioned working solutions to produce the calibration curve points equivalent to 2.0 (20 μl × 0.1 μg/ml), 5.0 (50 μl × 0.1 μg/ml), 10.0 (10 μl ×

1 μg/ml), 20.0 (20 μl × 1 μg/ml), 50.0 (50 μl × 1 μg/ml), 100.0 (10 μl × 10 μg/ml) and 300.0 (30 μl × 10 μg/ml) ng/ml of spironolactone and canrenone. Each sample also contained 20.0 ng (20 μl × 1 μg/ml) of the IS. In each run, a plasma blank sample (no IS) was also analyzed. Calibration curves were prepared by determining the best fit of the peak area ratios (peak area of analyte/peak area of IS) *versus* concentration, and fitted to the equation $R = aC + b$ by unweighted least-squares regression.

## Quality control samples

Quality control samples were prepared daily by spiking different samples of 1.0 ml blank plasma each with an appropriate volume of the corresponding standard solution to produce a final concentration equivalent to the low level (5.0 ng/ml), middle level (20.0 ng/ml) and high level (100.0 ng/ml) of spironolactone and canrenone with 20 ng/ml of the IS. The procedures were as described below.

## Sample preparation

QC, calibration curve and clinical plasma samples were extracted employing a liquid–liquid extraction technique. A 1.0 ml aliquot of the collected plasma sample from a human volunteer was pipetted into a 10 ml centrifuge tube. The working IS solution (20 μl × 1 μg/ml) and 5 ml methylene chloride : ethyl acetate mixture (20 : 80, v/v) were added and then vortexed for 2 min. Afterward, samples were centrifuged at 3000 rpm for 10 min. The organic layer was transferred to another 10 ml centrifuge tube and evaporated to dryness under a gentle stream of nitrogen gas in a water bath at 40 °C. The residue was redissolved in 100 μl of the mobile phase. An aliquot of 10 μl was injected into the LC-MS system.

## Method validation

The method validation assays were carried out according to the currently accepted U.S. Food and Drug Administration (FDA) bioanalytical method validation guidance.[13]

### *Assay specificity*

The specificity of the method was tested by analyzing blank plasma samples from six healthy humans. Each blank sample was tested for interference using the proposed extraction procedure and chromatographic/mass spectroscopic conditions and compared with those obtained with an aqueous solution of the analyte at a concentration near the lower limit of quantification (LLOQ).

The matrix effect on the ionization of the analytes was evaluated by comparing the peak areas of the analytes resolved in the blank sample (the final solution of blank plasma after extraction and reconstitution) with that resolved in the mobile phase. Three different concentration levels of spironolactone and canrenone (5.0, 20.0 and 100.0 ng/ml) and 20 ng/ml of the IS were evaluated by analyzing five samples at each level. The blank plasmas used in this study were six different batches of healthy human blank plasmas. If the ratio is <85% or >115%, an exogenous matrix effect is implied.

### *Linearity*

Calibration curves of seven concentrations of spironolactone and canrenone ranging from 2.0–300.0 ng/ml were extracted and assayed. Blank plasma samples were analyzed to confirm the absence of interferences but were not used to construct the calibration function. The lower limit of detection (LLOD) and the LLOQ were determined as the concentrations at signal-to-noise ratios of 3 and 10, respectively.

### *Precision and accuracy*

The precision of the assay was determined from the QC plasma samples by replicate analyses of three concentration levels of spironolactone (5.0, 20.0 and 100.0 ng/ml) and canrenone (5.0, 20.0 and 100.0 ng/ml). Within-batch precision and accuracy were determined by repeated analyses of the group of standards on one day ($n = 5$). Between-batch precision and accuracy were determined by repeated analyses on three consecutive days ($n = 5$ series per day). The concentration of each sample was determined using the calibration curve prepared and analyzed on the same day.

### *Extraction recovery*

The extraction recoveries of spironolactone and its active metabolite canrenone were determined at low, medium and high concentrations. Recoveries were calculated by comparing the analyte/I.S. peak area ratios ($R_1$) obtained from extracted plasma samples with those ($R_2$) from the standard solutions at the same concentration.

### *Stability*

Freeze and thaw stability: QC plasma samples at three concentration levels were stored at the storage temperature (−20 °C) for 24 h and thawed unassisted at room temperature. When completely thawed, the samples were refrozen for 24 h under the same conditions. The freeze–thaw cycles were repeated twice, and the samples were analyzed after three freeze (−20 °C)–thaw (room temperature) cycles.

Short-term temperature stability: QC plasma samples at three concentration levels were kept at room temperature for a period that exceeded the routine preparation time of the samples (around 6 h).

Long-term stability: QC plasma samples at three concentration levels kept at low temperature (−20 °C) were studied for a period of 4 weeks.

Postpreparative stability: The autosampler stability was conducted by reanalyzing extracted QC samples kept under autosampler conditions (4 °C) for 12 h.

Stock solution stability: The stability of spironolactone, canrenone and the IS working solutions were evaluated at room temperature for 6 h.

### *Standard curves and quality control samples in each batch*

Standard curves in each analytical run were used to calculate the concentrations of spironolactone and canrenone in the unknown samples in the run. They were prepared along with the unknown samples in the same batch and analyzed in the middle of the run.

The QC samples of spironolactone and canrenone in five duplicates at three concentrations (5.0, 20.0 and 100.0 ng/ml)

were prepared and were analyzed along with processed test samples at intervals in each batch.

## PRELIMINARY APPLICATION

Two healthy male Chinese volunteers participated in this preliminary pharmacokinetic study. After overnight fasting, each volunteer received tablets containing 100 mg spironolactone, which were taken with 200 ml water. Blood was sampled predose and at 0.25, 0.5, 0.75, 1, 1.5, 2.5, 3, 3.5, 5, 8, 12, 24, 36 and 60 h postdose for the determination of plasma concentrations of spironolactone and its metabolite canrenone.

## RESULTS AND DISCUSSION

### Selection of the ionization mode and mass conditions

In the early stage of the development of the method, attempts were made to use electrospray ionization (ESI) for spironolactone and canrenone analyses. Positive ion electrospray mass scan spectra of spironolactone, canrenone and the IS are shown in Figs 2, 3 and 4, respectively. The major ions observed were $[M - SCOCH_3]^+$, $m/z = 341.15$; $[M + Na]^+$, $m/z = 439.15$; $[M + K]^+$, $m/z = 455.15$ and $[M + Na + CH_3OH]^+$, $m/z = 471.25$ for spironolactone, $[M + H]^+$, $m/z = 341.10$; $[M + Na]^+$, $m/z = 363.10$; $[M + K]^+$, $m/z = 379.05$ and $[M + Na + CH_3OH]^+$, $m/z = 395.15$ for canrenone and $[M + H]^+$, $m/z = 294.95$; $[M + Na]^+$, $m/z = 316.95$; $[M + K]^+$, $m/z = 332.90$ and $[M + Na + CH_3OH]^+$, $m/z = 349.00$ for the IS. The $[M + Na]^+$ ion and $[M + Na + CH_3OH]^+$ ion dominated for spironolactone and canrenone, respectively. When these ions were selected for the determination of spironolactone and canrenone in human plasma, it was found that the sensitivity was not enough and the regression curves were nonlinear, which might have resulted from the instability of these adduct ions.

Therefore, APCI was tried. In the APCI full-scan spectra of canrenone and the IS, the $[M + H]^+$ ion dominated (Figs 5 and 6) with obviously increased intensity. For spironolactone, the fragment ion $[M - SCOCH_3]^+$ dominated (Fig. 7) with higher intensity and stability. So the ions of $[M - SCOCH_3]^+$, $m/z = 341.25$ for spironolactone; $[M + H]^+$, $m/z = 341.25$ for canrenone and $[M + H]^+$, $m/z = 295.05$ for IS were selected for the selected-ion monitoring (SIM)(+) because of their high intensity and stability.

Nebulizer gas flow rate, APCI temperature, CDL temperature and block temperature were all optimized in order to obtain the highest intensity of the analyte response under the developed mobile phase conditions. The other MS parameters were adopted from the recommended values for the instrument.

### Selection of IS

It is necessary to use an IS to get high accuracy when a mass spectrometer is used as the HPLC detector. Estazolam was

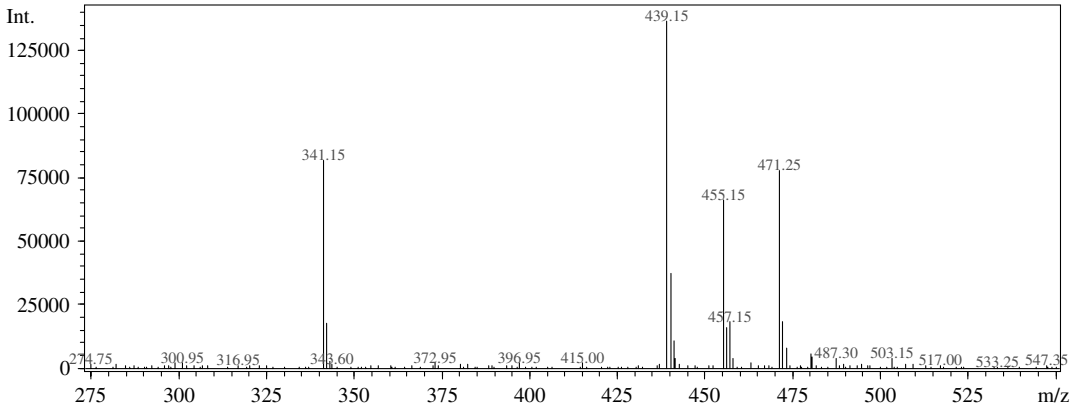


**Figure 2.** Positive ion electrospray mass scan spectrum of spironolactone.

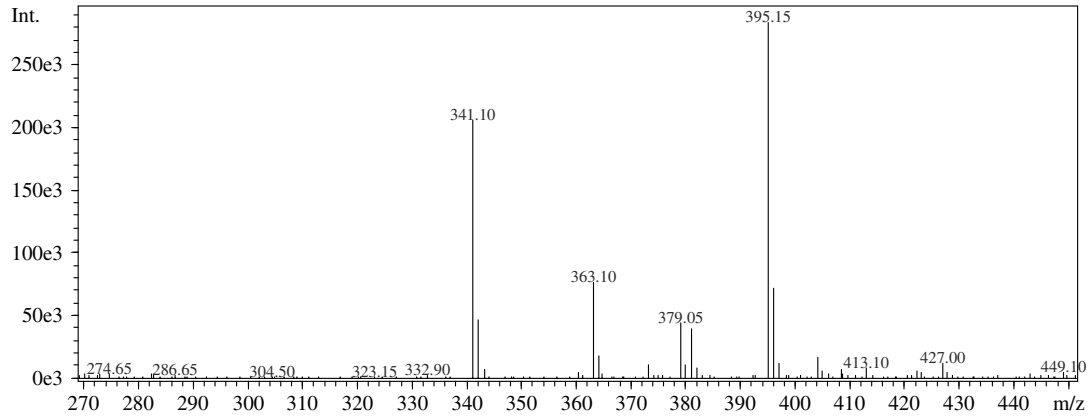


**Figure 3.** Positive ion electrospray mass scan spectrum of canrenone.

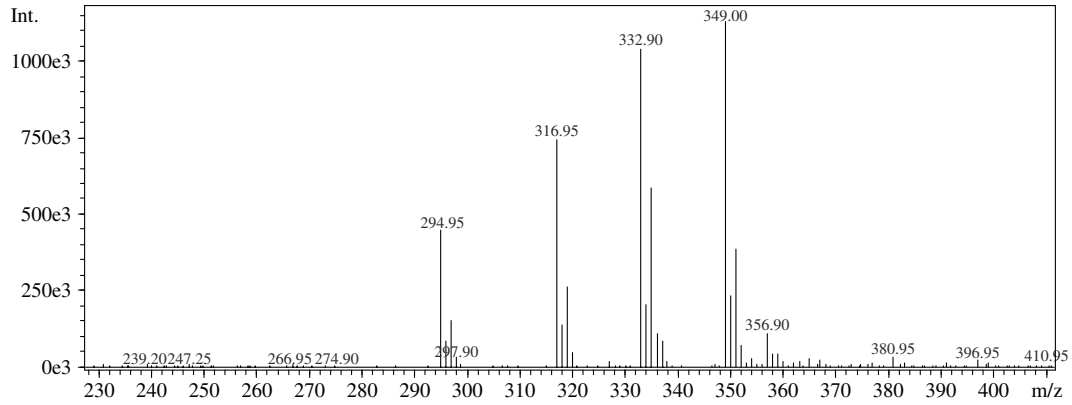


**Figure 4.** Positive ion electrospray mass scan spectrum of estazolam.

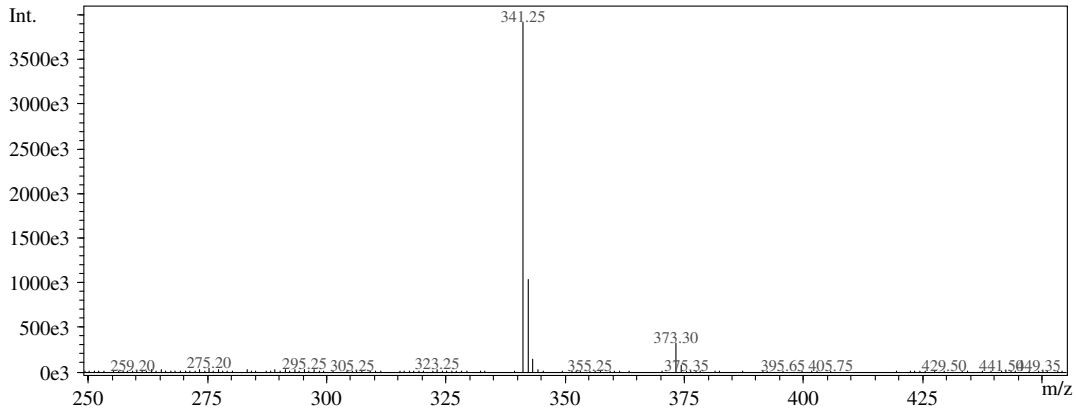


**Figure 5.** Positive ion APCI-mass scan spectrum of canrenone.

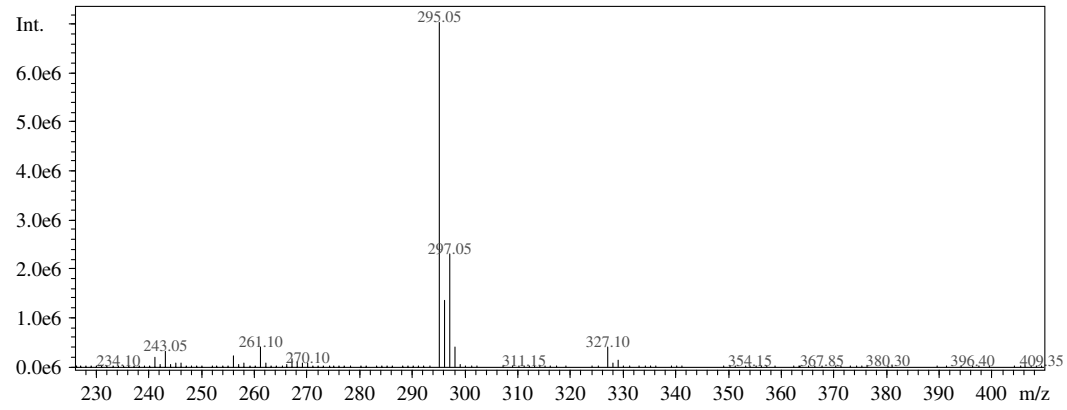


**Figure 6.** Positive ion APCI-mass scan spectrum of estazolam.

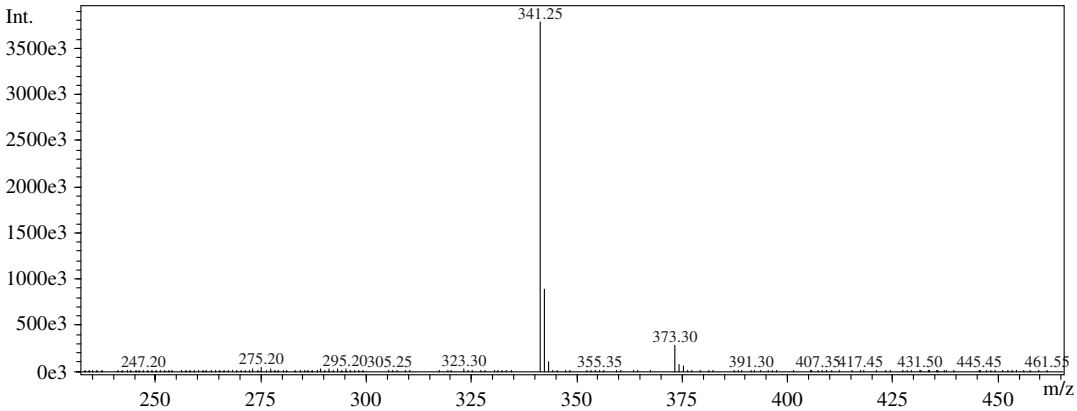


**Figure 7.** Positive ion APCI-mass scan spectrum of spironolactone.

adopted in the end because of the similarity of its retention time, ionization and extraction efficiency with the analytes, as well as the less endogenous interference at $m/z$ 295.05. The structure of estazolam is shown in Fig. 1.

## Sample preparation

Liquid–liquid extraction was important because this technique can not only purify but also concentrate the sample. Ethyl acetate, *n*-hexane–isopropanol (95 : 5, v/v) and methylene chloride–ethyl acetate mixture (20 : 80, v/v) were all attempted, and methylene chloride–ethyl acetate mixture (20 : 80, v/v) was finally adopted because of its high extraction efficiency.

## Separation

The SIM(+) chromatograms extracted from supplemented plasma are shown in Fig. 8. As shown, the retention times of spironolactone, canrenone and the IS were 4.4, 9.6 and 12.4 min, respectively.

The total HPLC-MS analysis time was 13.5 min per sample. A representative selected-ion chromatogram of a plasma sample obtained at 0.5 h from a subject who received a single oral dose (100 mg) is shown in Fig. 9.

## Method validation

### *Assay specificity*

No interferences of the analytes were observed because of the high selectivity of the SIM mode. All the ratios of the peak area resolved in the blank sample, compared with that resolved in the mobile phase, are between 85 and 115%, which means no matrix effect is observed for spironolactone, canrenone and estazolam in this method. Figure 10 shows an HPLC chromatogram for a blank plasma sample indicating no endogenous peaks at the retention positions of spironolactone, canrenone or the IS (estazolam).

### *Linearity and LLOQ*

The calibration curves of the analytes showed good linearity in the range 2.0–300.0 ng/ml for spironolactone and canrenone. The mean regression equations from five replicate calibration curves on different days were: $R = 0.0309C + 0.0019$, $r = 0.9993$ for spironolactone and $R = 0.0386C + 0.0069$, $r = 0.9994$ for canrenone, where $R$ corresponds to the peak area ratio of spironolactone or canrenone to the IS, and $C$ refers to the concentration of spironolactone or canrenone added to plasma. Results of the calibration curves for spironolactone and canrenone LC-MS determination are given in Table 1.

The lower limits of quantification for spironolactone and canrenone were proved to be 2 ng/ml (LLOQ) and the lower limits of detection (LLOD) were 1 ng/ml. Figure 11 shows the chromatogram of an extracted sample that contained 2 ng/ml (LLOQ) of spironolactone and canrenone.

### *Precision and accuracy*

Data for within-batch and between-batch precision and accuracy of the method for determination of spironolactone and canrenone are presented in Table 2. The accuracy deviation values are within 10% of the actual values. The precision determined at each concentration level does not

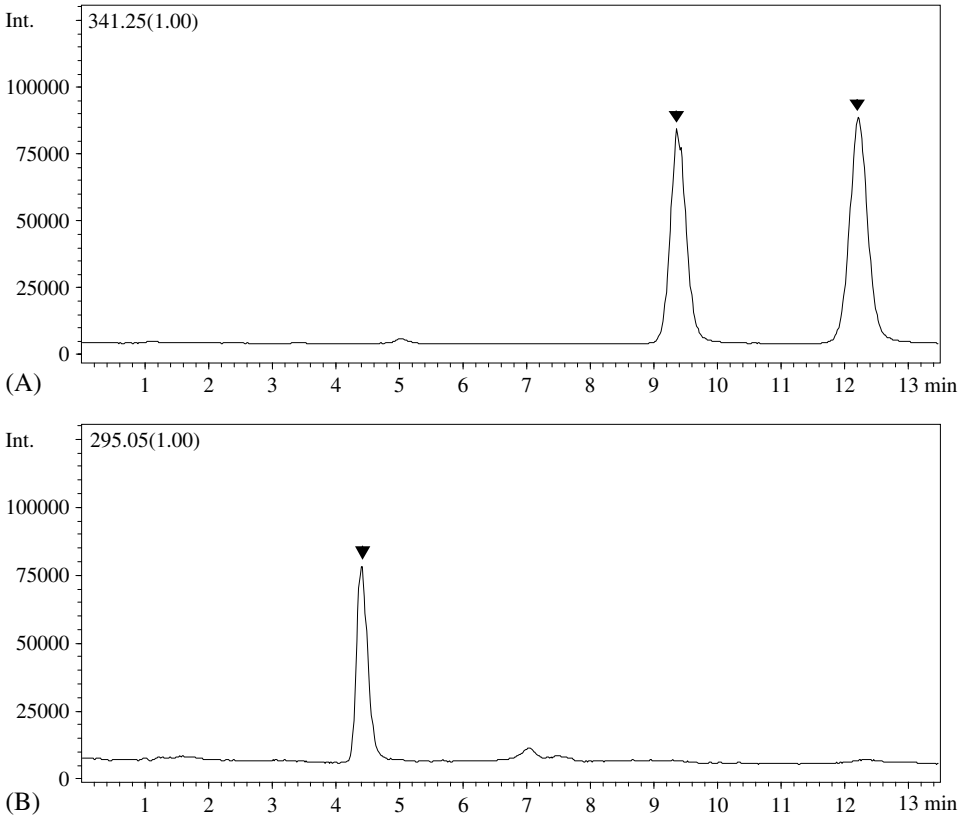


**Figure 8.** The SIM(+) chromatograms extracted from supplemented plasma. Peaks are assigned with ▼. The retention times of spironolactone and canrenone were 9.6 min and 12.4 min (A). The retention time of the IS was 4.4 min (B).

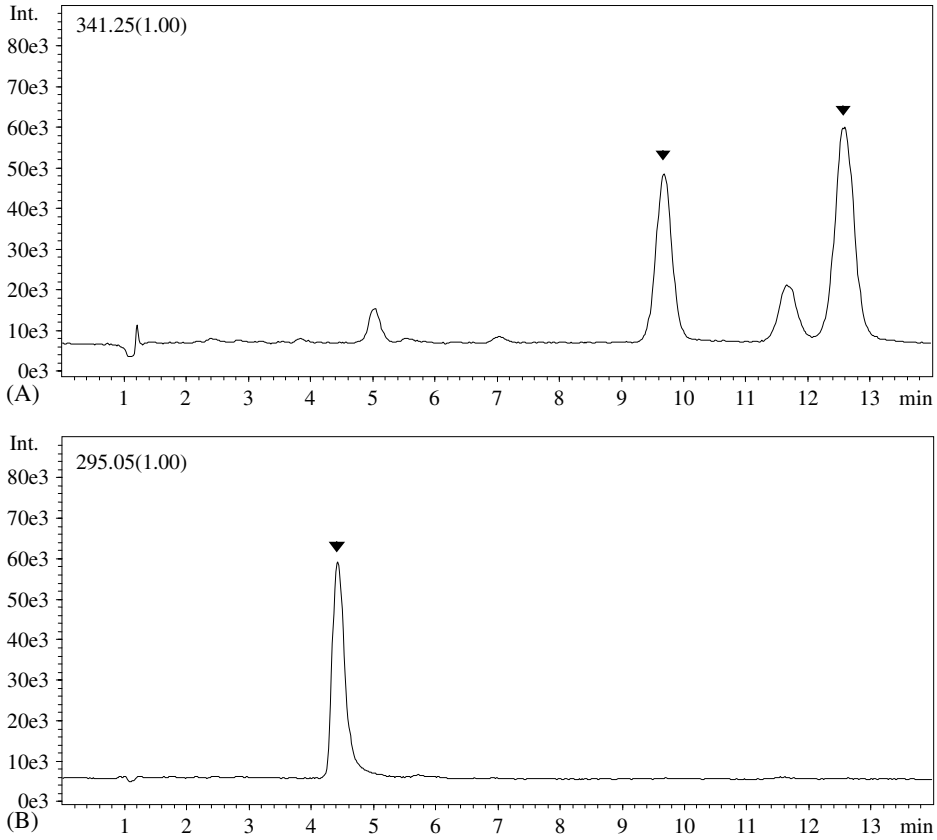


**Figure 9.** The SIM(+) chromatogram for the plasma sample of a healthy volunteer. Peaks are assigned with ▼. The retention times of spironolactone and canrenone are 9.6 min and 12.4 min (A). The retention time of the IS is 4.4 min (B).

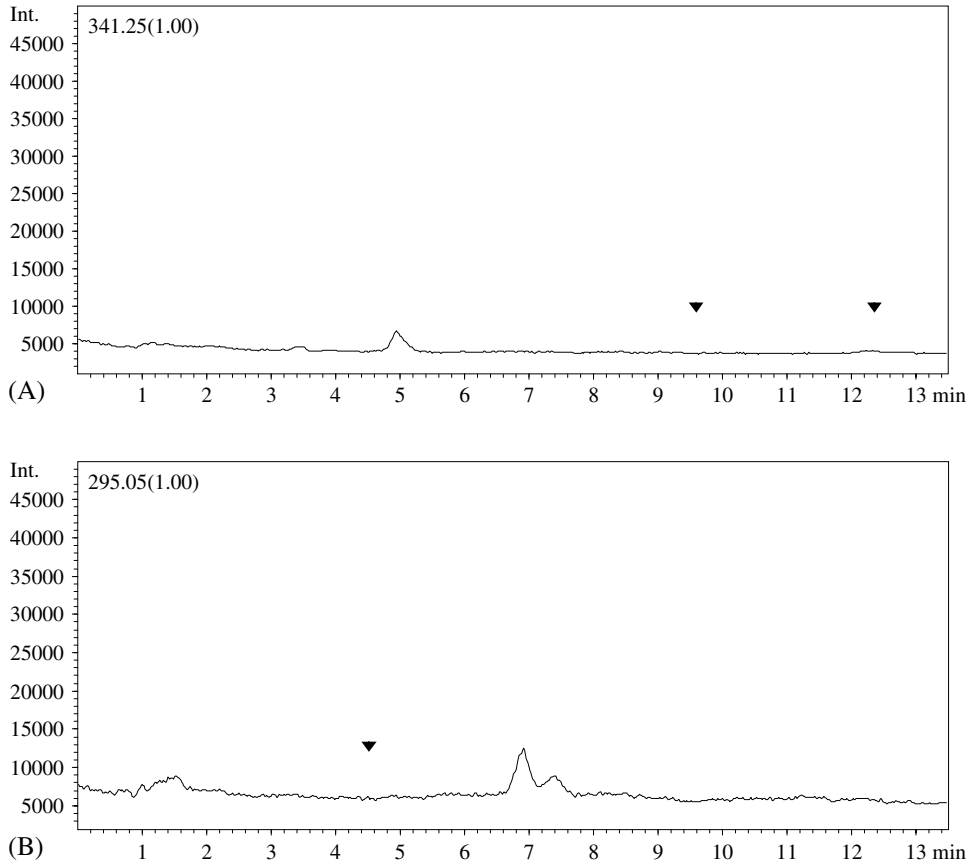


**Figure 10.** The SIM(+) chromatogram for a blank plasma sample. Peaks are assigned with ▼. The retention times of spironolactone and canrenone are 9.6 min and 12.4 min (A). The retention time of the IS is 4.4 min (B).

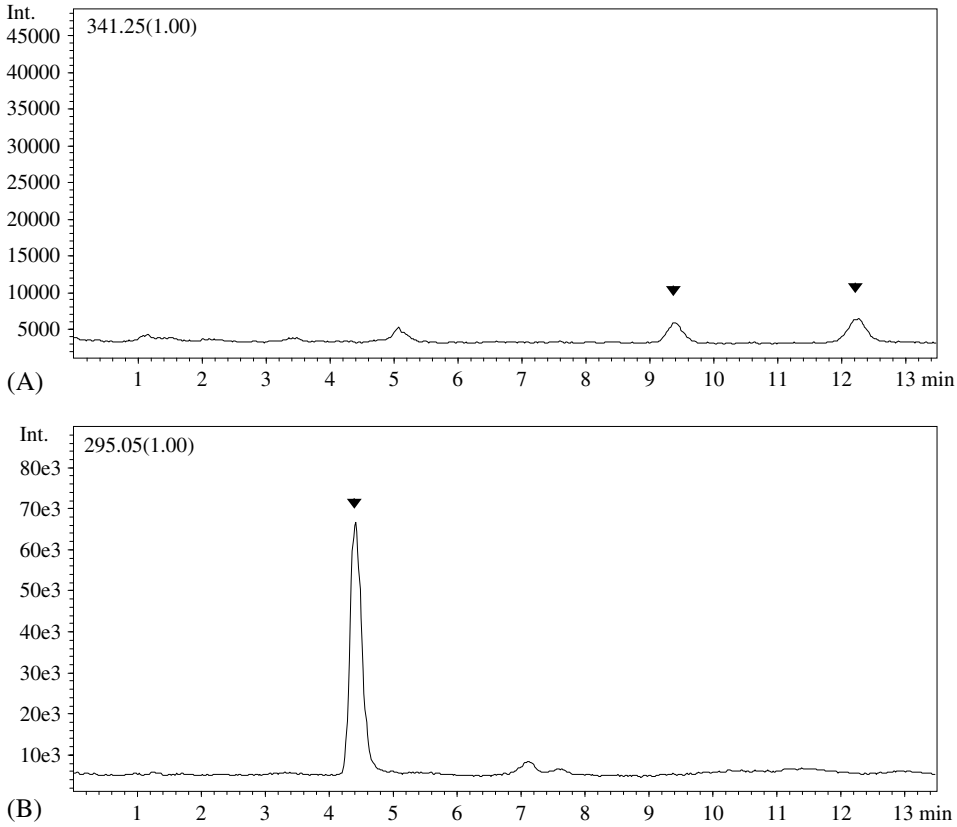


**Figure 11.** The SIM(+) chromatogram of LLOQ (2 ng/ml). Peaks were assigned with ▼.The retention times of spironolactone and canrenone are 9.6 min and 12.4 min (A). The retention time of IS is 4.4 min (B).

**Table 1.** Results of calibration curves for spironolactone and canrenone LC-MS determination ($n = 5$)

| | Spironolactone | | | Canrenone | | |
|---|---|---|---|---|---|---|
| Nominal (ng/ml) | Mean | RSD (%) | Accuracy (%) | Mean | RSD (%) | Accuracy (%) |
| 2.0 | 2.06 | 8.33 | 102.76 | 1.99 | 8.80 | 99.44 |
| 5.0 | 4.68 | 6.65 | 93.64 | 5.15 | 7.42 | 103.08 |
| 10.0 | 9.83 | 6.10 | 98.31 | 9.70 | 6.31 | 96.97 |
| 20.0 | 20.00 | 4.23 | 99.99 | 20.14 | 5.35 | 100.72 |
| 50.0 | 50.91 | 3.01 | 101.82 | 48.14 | 3.47 | 96.28 |
| 100.0 | 102.25 | 2.76 | 102.25 | 99.03 | 2.63 | 99.03 |
| 300.0 | 301.92 | 2.40 | 100.64 | 311.90 | 1.85 | 103.97 |

exceed 10% of the relative standard deviation (RSD). The results revealed good precision and accuracy.

### *Extraction recovery*

The extraction recoveries determined for spironolactone and canrenone were shown to be consistent, precise and reproducible. Data are shown in Table 3. The extraction recovery of IS was more than 85%.

### *Stability*

Table 4 summarizes the freeze and thaw stability, short-term stability, long-term stability and postpreparative stability data of spironolactone and canrenone. All the results showed

**Table 2.** The within- and between-batch precision and accuracy of the method for determination of spironolactone and canrenone (within batch: $n = 5$; between batch: $n = 15$ series per day)

| | | Spironolactone | | | Canrenone | | |
|---|---|---|---|---|---|---|---|
| Added conc. (ng/ml) | | Detected conc. (mean ± SD, ng/ml) | Mean accuracy (%) | RSD (%) | Detected conc. (mean ± SD, ng/ml) | Mean accuracy (%) | RSD (%) |
| | 5.0 | 4.85 ± 0.34 | 96.91 | 7.04 | 5.10 ± 0.33 | 102.01 | 6.50 |
| Within batch | 20.0 | 19.08 ± 0.98 | 95.38 | 5.13 | 20.43 ± 0.94 | 102.14 | 4.59 |
| | 100.0 | 98.08 ± 3.50 | 98.08 | 3.57 | 100.38 ± 2.99 | 100.38 | 2.98 |
| | 5.0 | 4.89 ± 0.31 | 97.77 | 6.38 | 5.11 ± 0.30 | 102.17 | 5.89 |
| Between batches | 20.0 | 19.54 ± 1.04 | 97.68 | 5.30 | 20.26 ± 0.79 | 101.29 | 3.91 |
| | 100.0 | 98.72 ± 2.72 | 98.72 | 2.75 | 99.56 ± 2.56 | 99.56 | 2.57 |

**Table 3.** Recovery of spironolactone and canrenone from plasma ($n = 5$)

| | Spironolactone | | Canrenone | |
|---|---|---|---|---|
| Added conc. (ng/ml) | Recovery (mean ± SD, %) | RSD (%) | Recovery (mean ± SD, %) | RSD (%) |
| 5.0 | 92.52 ± 5.22 | 5.64 | 94.24 ± 4.84 | 5.13 |
| 20.0 | 91.68 ± 2.80 | 3.06 | 91.40 ± 3.35 | 3.67 |
| 100.0 | 90.72 ± 2.49 | 2.75 | 91.27 ± 1.14 | 1.25 |

good stability during these tests and there were no stability-related problems during the routine analysis of samples for pharmacokinetic, bioavailability or bioequivalence studies.

The stability of the working solutions was tested at room temperature for 6 h. On the basis of the results obtained, these working solutions were found to be stable within 6 h.

*Application*

The method described above was successfully applied to a preliminary pharmacokinetic study in which plasma concentrations of spironolactone and its metabolite canrenone were determined for up to 60 h after administration of tablets containing 100 mg spironolactone. The pharmacokinetic profiles of spironolactone and canrenone from two subjects after oral administration are shown in Fig. 12.

## CONCLUSIONS

The described method achieved fairly good sensitivity and specificity for the simultaneous determination of spironolactone and its active metabolite canrenone in human plasma. The simple liquid–liquid extraction procedure and the short run time can curtail test time, which is important for large batches of samples. This simple and rapid assay is suitable for pharmacokinetic, bioavailability or bioequivalence studies of spironolactone and its metabolite canrenone in human subjects.

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**Table 4.** Data showing the stability of spironolactone and canrenone in human plasma at different QC levels ($n = 5$)

| | Accuracy (mean ± SD, %) | | | | | |
|---|---|---|---|---|---|---|
| | Spironolactone | | | Canrenone | | |
| | 5.0 (ng/ml) | 20.0 (ng/ml) | 100.0 (ng/ml) | 5.0 (ng/ml) | 20.0 (ng/ml) | 100.0 (ng/ml) |
| Freeze and thaw stability | 97.05 ± 6.86 | 98.12 ± 3.94 | 98.68 ± 3.47 | 102.88 ± 5.36 | 96.96 ± 4.36 | 99.82 ± 2.73 |
| Short-term stability | 94.12 ± 6.95 | 98.70 ± 5.16 | 101.24 ± 3.18 | 93.76 ± 8.52 | 97.28 ± 5.71 | 102.34 ± 3.20 |
| Long-term stability | 92.45 ± 7.23 | 95.06 ± 4.53 | 94.19 ± 2.68 | 94.87 ± 6.59 | 97.87 ± 5.92 | 95.42 ± 2.99 |
| Postpreparative stability | 96.58 ± 5.41 | 97.33 ± 4.27 | 98.55 ± 3.04 | 104.53 ± 7.42 | 99.89 ± 6.37 | 96.28 ± 2.57 |

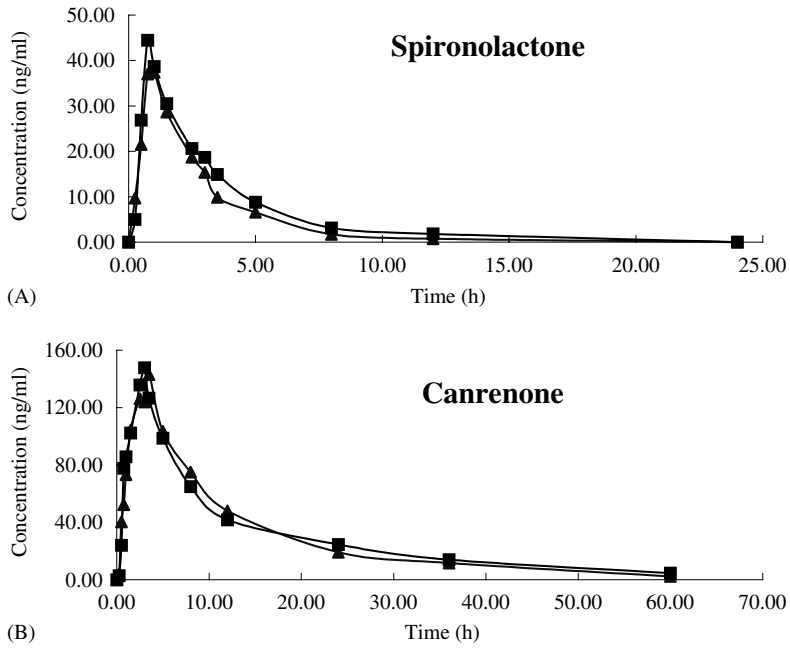


**Figure 12.** The pharmacokinetic profiles of spironolactone (A) and canrenone (B) from two subjects after oral administration.

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