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Simple and rapid liquid chromatography–tandem mass spectrometry method for simultaneous determination of (S)-allyl-L-cysteine and its metabolites in rat plasma: application to pharmacokinetic study in rats

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Garlic has been used worldwide for food and folk medicine for a long time. Garlic contains various kinds of organosulfur compounds, which shows health benefits of garlic [1]. Among these, (S)-allyl-L-cysteine (SAC) has potent pharmacological activity and low toxicity [2]. As other phytochemicals, metabolism of SAC is occurred rapidly in the body. SAC is metabolized to N-acetyl-(S)-allyl-L-cysteine (NASAC) by N-acetyl transferase and NASACS is metabolized to N-acetyl-(S)-allyl-L-cysteine sulfoxide (NASACS) by flavin-containing monohydrogenases (FMOs). Alternatively, SAC is also metabolized to (S)-allyl-L-cysteine sulfoxide (SACS) by FMOs and metabolized to NASACS by N-acetyl transferase in rat, rabbit and Human (Fig. 1) [3].

In this study, a simple liquid chromatography–tandem mass spectrometry method was developed and validated in rat plasma for simultaneous quantification of SAC and its metabolites for pharmacokinetic study. The chromatographic separation was performed on a reverse-phase C18 column with a mobile phase consisting of 0.1% formic acid and acetonitrile at a flow rate of 0.3 mL min^{-1}. The protonated analyte was quantified by multiple reactions monitoring with a Waters Quattro microTM API mass spectrometer (Fig. 1). The calibration curves of SAC, SACS, NASAC, and NASACS were linear over each concentration range, and the lower limits of quantification were 0.1 μg mL^{-1} (SAC and NASAC) and 0.25 μg mL^{-1} (SACS and NASACS), with precisions (coefficient of variation (CV)%) of 6.1% (SAC), 5.0% (SACS), 4.4% (NASAC), and 4.1% (NASACS) rat plasma samples. Acceptable intra-day and inter-day precisions and accuracies were obtained at three concentration levels. Acceptable intra-day and inter-day precisions and accuracies were obtained at three concentration levels. SAC, SACS, NASAC, and NASACS were found to be stable in a battery of studies, including bench-top, freeze–thaw, and autosampler conditions. The validated method was successfully used to determine SAC, SACS, NASAC, and NASACS concentrations in rat plasma samples after administration at oral doses of 50 mg kg^{-1}.

Keywords: (s)-allyl-l-cysteine; LC-MS/MS; Absorption; Metabolism; Garlic; Pharmacokinetics

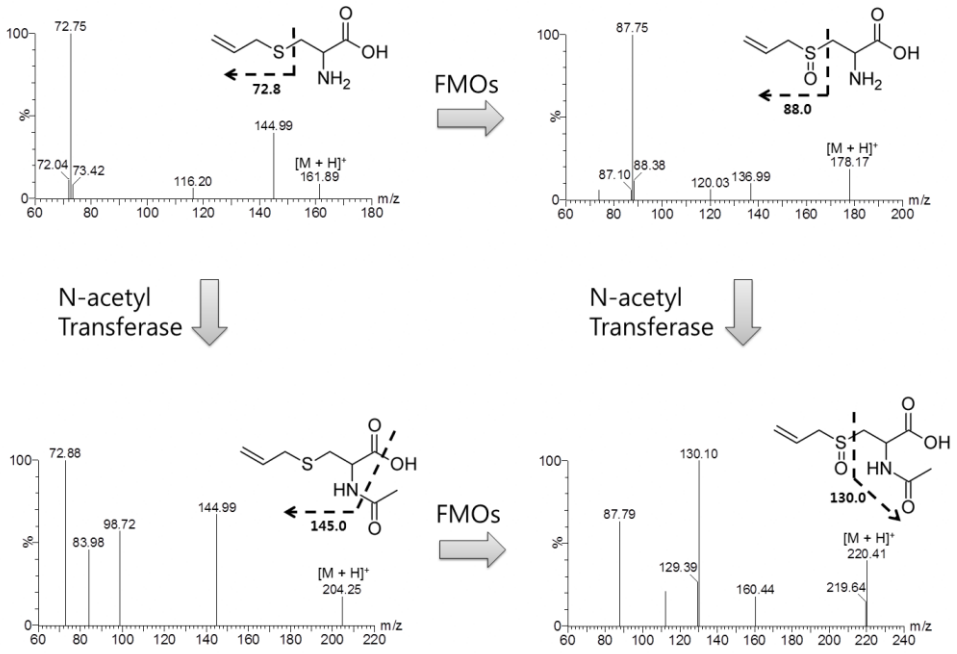

Fig.1. MS spectra and fragment pattern of SAC (A), SACS (B), NASAC (C), NASACS (D).

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References

[1] Amagase H, Petesch BL, Matsuura H, et al. Intake of garlic and its bioactive components 2001, J Nutr. 131:955S-962S

[2] Nakagawa S, Masamoto K, Sumiyoshi H, et al. Acute toxicity test of garlic extract. 1984, J Toxicol. Sci. 9:57-60.

[3] Krause R J, Glocke S C, Elfarra A A. Sulfoxides as urinary metabolites of *S*-allyl-L-cysteine in rats: evidence for the involvement of flavin-containing monooxygenases. 2002, Drug Metab Dispos 30:1137-1142.