

# Simultaneous LC-MS/MS quantitation of sulforaphane and its metabolites: Investigation of sulforaphane metabolism in rat tissues

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## Overview

- Sulforaphane (SFN) and four of its metabolites were studied in various rat tissue samples using LC-MS/MS.
- The sulforaphane conjugates were synthesized, purified and used as external standards in MRM quantitation analysis.
- Three different sample preparation techniques were tested for best analyte recovery from tissue samples.
- An unknown potential SFN metabolite was investigated

## Introduction

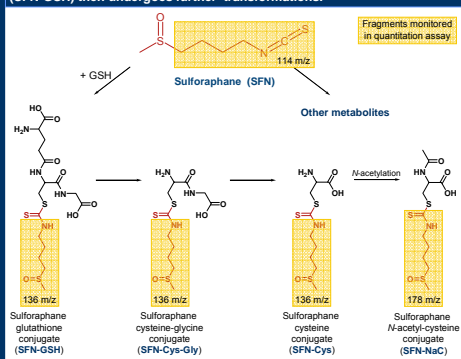
Isothiocyanates, such as sulforaphane (SFN), which naturally occur in cruciferous plants, are a candidate class of cancer preventive compounds<sup>1</sup>. Studies show that they stimulate the body's production of detoxification enzymes (phase 2), inhibit carcinogenesis, and exert antioxidant effects. *In vivo*, SFN reacts with various compounds, including glutathione (GSH), to produce a number of conjugated metabolites. In ongoing studies we focus on determining the SFN metabolic path *in vivo* by analyzing various tissue samples from rats fed with synthetic SFN. A sensitive and reliable quantitative method for analysis of SFN and its metabolites has been developed.

## Methods

**Quantitation assay:** Four sulforaphane conjugates, synthesized and purified in-house<sup>2</sup>, were used along with commercially available sulforaphane as external standards to build calibration curves. Data acquisition was performed in multiple-reaction monitoring (MRM) mode, using unique parent ion-daughter ion transitions for each compound (Fig.1)

### SCHEME 1. Sulforaphane and its metabolites

Following ingestion, sulforaphane reacts with various compounds including glutathione (GSH). The sulforaphane-glutathione conjugate (SFN-GSH) then undergoes further transformations.



**Sample preparation:** Eight-week-old rats were gavaged-fed with SFN for five days; 24 hours after last feeding, various tissue samples, including liver, kidney, stomach, and serum were harvested. Samples were homogenized (3 different methods, see Fig. 2), ultrasonicated, and subjected to protein precipitation in cold acetonitrile at acidic pH. Solid phase extraction on C18 cartridges was utilized to further decrease the sample complexity.

**Instrumentation:** Analysis was carried out by ESI LC-MS/MS using an Agilent 1100 HPLC and Micromass Quattro Premier triple quadrupole mass spectrometer. QuanLynx software was used for data analysis. HPLC conditions: C18 Luna 150 x 2.1 mm column, 3µm particles; 2-80% B gradient in 20 min., total run time 29 min.; A: 0.1% formic acid in water, B: 0.1% formic acid in acetonitrile; 250 µl/min flow rate.

## Results

FIGURE 1. MRM transitions for sulforaphane and conjugates

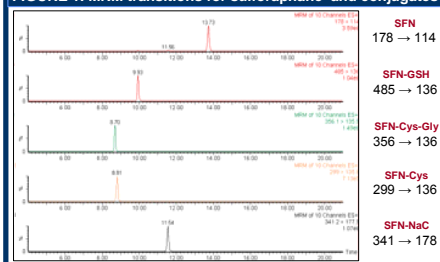


FIGURE 2. Sample preparation studies

Rat liver tissue was used in sample preparation studies in which three different protocols were tested for best recovery of SFN metabolites. Tissue samples were homogenized and ultrasonicated in either 20 mM ammonium acetate (AA), 0.1% formic acid (FA) or 5% sulfosalicylic acid (SSA). Criteria for method evaluation: analyte recovery, signal-to-noise ratio (S/N), and sample matrix interferences. The AA procedure was chosen for use in further analyses.

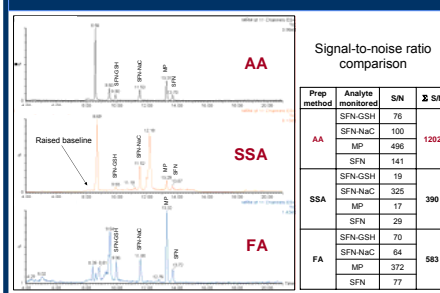


FIGURE 3. Analysis of tissue samples

Rat liver, kidney, and stomach samples were prepared using the AA extraction protocol. Serum samples underwent protein precipitation in cold acidic acetonitrile. Organs from three animals were harvested and analyzed separately. Significant differences in analyte abundances were observed among the various tissue types.

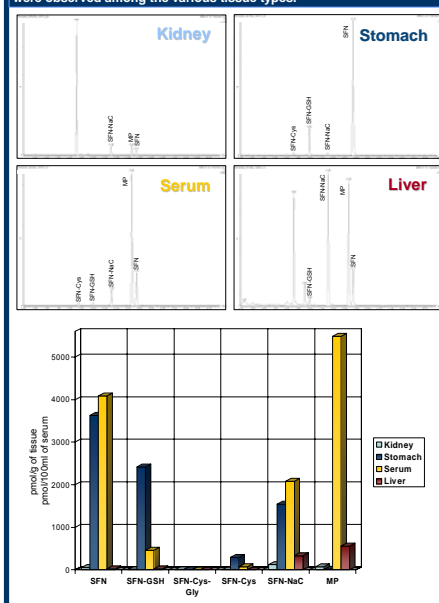


FIGURE 4. Unknown metabolite investigation: MRMs

Potential SFN metabolite peak (MP) was detected at RT= 13.3 min in all types of tissue from SFN fed rats.\* MP shares four transitions with SFN, which suggests that it is a sulforaphane derivative.

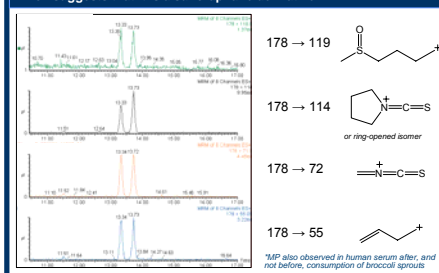
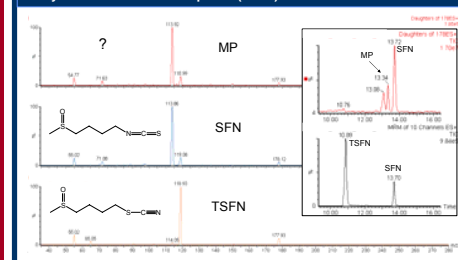


FIGURE 5. Unknown metabolite investigation: daughter ions MS/MS spectra of the 178 m/z parent ion were acquired from a sample containing both SFN and MP. It was established that MP is not the thiocyanate isomer of sulforaphane (TSFN)<sup>3</sup>



## Conclusions

- A reliable quantitation assay was established for simultaneous monitoring of SFN and its conjugates. Linear response of calibration curve was observed from 2 fmol to 200 pmol for the conjugates and from 2 fmol to 10 pmol for sulforaphane.
- Use of ammonium acetate buffer gives most satisfactory data quality and analyte recovery from tissue samples.
- Various metabolite distributions among different tissues were observed.
- An unknown potential SFN metabolite was observed and partially characterized.

## Future work

- Compare levels of sulforaphane metabolites in normal and tumor samples
- Extend study to human tissue samples, comparing SFN metabolite levels in healthy and tumor tissues
- Further characterization of unknown sulforaphane metabolites

## References

- Zhang, Y.; Talalay, P. *Proc. Natl. Acad. Sci.* **1992**, *89*, 2399-2403.
- Matusheski, N.; Wallig, M.; et al. *J. Agric. Food Chem.* **2001**, *49*, 1867-1872.
- Kassahun, K.; Davis, M.; et al. *Chem. Res. Toxicol.* **1997**, *10*, 1228-1233.
- Jones, S.; Brooks, J.D. *BMC Cancer* **2006**, (6) 62.

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