Characterization of nucleotide and nucleoside analogs from Taraxacum mongolicum, a Chinese medicinal herb was achieved for the first time using liquid chromatography tandem mass spectrometry (LC/MS/MS). The active ingredients in the herb were characterized using nucleotide and nucleoside standards with product and precursor scans of tandem mass spectrometry. The nucleoside analogs were believed to be major active ingredients which have anti-Herpes simplex virus type 1 properties. While other methods are needed for the positive compound identification, the LC/MS/MS does provide a valuable tool for the characterization of active ingredients in natural products.

**Introduction**

Many emerging viruses pose threats to our world as we enter the twenty-first century. The association of viruses with many life-threatening diseases makes it necessary to examine variform treatments for viral infections. Herbal medicines are currently being sought after for viral diseases because customary treatments are not proving very effective. We have shown that the aqueous and methanolic herbal extracts of the Chinese herb Taraxacum mongolicum have anti-Herpes simplex virus type-1 properties *in vitro*. Using liquid chromatography tandem mass spectrometry (LC/MS/MS) approaches and other spectroscopic and chromatographic techniques, we have tentatively identified the active ingredients as nucleoside analogs. Currently there are only a few classes of drugs with anti-HSV-1 properties, including nucleotide and nucleoside analogs. LC/MS/MS conditions were optimized using several nucleotide and nucleoside drug standards that are commercially available. Precursor and product ion scan modes were used to screen nucleotide and nucleoside analogs in the herbal extract. Three nucleoside analogs were found in Taraxacum mongolicum and their chemical structures were proposed.
Method/experimental conditions

Sample Preparation
- 10 g dried *Taraxacum mongolicum* boiled in 100 mL methanol for 45 minutes
- Liquid subsequently filtered with Whatman grade filter paper
- Methanol roto-evaporated, leaving residue
- Residue reconstituted in mobile phase to place on LC/MS

LC Conditions
- Column: YMC C18, 2.1x250 mm, 5 µm
- Flow rate: 300 µl/min
- Mobile phase: A-acetonitrile, B-2 mM ammonium acetate
- Gradient: 5-60% ACN in 10 min and hold for 2 min
- Injection: 10 µl
- Autosampler: PE series 200

MS Conditions
- API 2000™ LC/MS/MS System triple quadruple mass spectrometer
- Ion source: TurbolonSpray® source, positive & negative modes
- Scan modes: Product and precursor scans

Study design
There are three major chemical analogs that have anti-viral activities: protease inhibitors, nucleotide, and nucleoside analogs. Protease inhibitors are synthetic compounds while nucleotide and nucleoside analogs are found in natural products.

To screen nucleotides: Four nucleotide standards in adequate solutions were infused into MS/MS for optimization of the instrument. The common...
product ions of 159 and 79 m/z were used for the precursor scans of potential nucleotide analogs in the crude extract samples under the same LC/MS/MS conditions for nucleotide standards.

To screen nucleosides: Three nucleoside standards were tested under optimized MS/MS conditions. The most abundant product ion was the base fragment for all three nucleoside standards. Known nucleoside structures were searched and all base fragments were calculated. These base fragments were used for precursor ion scans of potential nucleoside analogs in the crude extract under optimized LC/MS/MS conditions for nucleoside standards.

**Conclusions**

- This method shows a successful example of using the LC/MS/MS methodology for identifying the presence of nucleotide and nucleoside analogs in crude herbal extract samples. This technology can be used as a general tool to characterize other natural products.
- The extraction method was simple and effective. Following a rational study design, the powerful LC/MS/MS method allows a direct injection of a crude herbal extract to characterize active ingredients such as nucleoside and nucleotide analogs in herbs.
- The results demonstrated that there may be at least three nucleoside analogs present in the herbal extract of Taraxacum mongolicum. Since this herb has anti-Herpes simplex virus type-1 properties, the nucleoside analogs may be responsible for the anti-viral activities. To positively identify the chemical structures, more experiments are being conducted, such as NMR and Qq-TOF for detailed structure elucidation and exact molecular weight measurement. The identified peaks will also be tested for anti-viral activities *in vitro.*

![Figure 4. MS/MS Spectra and precursor ion scans of nucleoside standards and crude herb extract.](image-url)
Figure 5. Precursor ion scans of three nucleoside standards and proposed nucleosides in herb.