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Quantitation of Oleanolic Acid in Rat Plasma Using Modifier-assisted Differential Mobility Spectrometry Tandem Mass Spectrometry Coupled with Multiple Ion Monitoring

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Research Article

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ABSTRACT

Bioanalysis of oleanolic acid in rat plasma using Differential Mobility Spectrometry (DMS) tandem mass spectrometry combined with Multiple Ion Monitoring (MIM) method was developed and validated. Oleanolic acid, a kind of pentacyclic triterpenoid compound derived from plants, has various biological activities. It is difficult to determine using LC-MS/MS because it has poor Collision-Induced Dissociation (CID) efficiency when analyzing in Multiple Reaction Monitoring (MRM) mode. In order to increase the sensitivity and simplify the biological sample preparation, we explored a UPLC-DMS-MIM approach. Good accuracy and precision were achieved in the range 3-300 ng/mL with a run time of only 3 min. Isopropanol as organic modifier facilitated a good combination of sensitivity and separation. The method is a proof-of-principle report of the analysis of compounds that unable to generate stable and abundant fragment due to too much fragmentation or poor CID efficiency.

INTRODUCTION

Oleanolic Acid (OA) is a plant-derived pentacyclic triterpenoid compound with various biological activities, widely found in fruits and vegetables [1]. It has wild clinical application, including inhibitory of cancer [2-4], anti-osteoporosis [5], protection against gastrointestinal diseases [6], improvement of diet-induced obesity [7], lipid-lowering [8], anti-inflammatory [9], antioxidant and immune-regulatory [10], epatoprotective effects [11] OA tablets has been applied to clinical adjuvant treatment of acute and chronic hepatitis as an over the counter drug for decades in China. Recent researches on OA are mainly focus on the exploration of new pharmacological effects, *in vivo* pharmacodynamic mechanism, development of different OA dosage forms. Moreover, OA could serve a framework for the development of novel semi-synthetic triterpenoids. All above needs the support of PK research *in vivo.*

At present, liquid chromatography-mass spectrometry combined with Multiple Reaction Monitoring (MRM) has become the most widely used method to analyze samples in biological matrix [12]. In the MRM mode of a triple quadrupole mass spectrometer, the first quadrupole (Q1) scans the precursor ion, the second quadrupole (Q2) produces one or more major fragment ions through collision induced dissociation (CID), and the third quadrupole (Q3) scans the product ions. Therefore, the sensitivity of MRM depends not only on the abundance of precursor and product ions, but also on the efficiency of CID. The inefficient CID fragmentation issues have challenged the sensitivity of MRM. Because OA belongs to pentacyclic triterpenes, it is difficult to be fragmented and to generate abundant fragment ions for MRM detection in quadrupole 3. Several LC-MS or LC-MS/MS methods have been reported to determine OA in biological fluids. Zheng, et al. designed and synthesized a derivatization reagent to extract OA from rat blood using magnetic dispersive solid phase extraction before LC-MS/MS [13]. Kim, et al. developed an LC-MS/MS method after liquid-liquid extraction with ethylacetate and polyethylene to determine 3-O-acetyl-OA and OA in rat plasma [14]. Besides the long sample preparation time, the analysis time was seven minutes per run while the retention time for OA was 4.44 min. Zhao, et al. developed a 13-minute LC-MS method to quantify oleanolic and ursolic acids in the rat plasma using HPLC-MS after liquid extraction [15]. Shi, et al. did a pharmacokinetic study of OA in rat using liquid chromatography-tandem mass spectrometry (LC-MS/MS) under Selected Ion Monitoring (SIM) mode [16].

The unavoidable disadvantages of using LC-MS or LC-MS/MS are: 1) low ionization efficiency, resulting in low sensitivity, so that a derivatization step has to be used to improve ionization efficiency; 2) low cracking efficiency using Multiple Ion Monitoring (MIM) [17]. Although MIM is improved on the basis of SIM, but it is better than SIM under the same chromatographic conditions, but the interference still exists. Under MIM mode, the same m/z is selected for both Q1 and Q3. Q1 selected the most abundant precursor ion and Q3 let the intact precursor ion pass through to the detector. In Q2, the priority selections for collision energy are to obtain the minimum analyte intensity loss and the minimum isobaric matrix effect. Due to the potential isobaric ions of the same m/z generated from the biological matrices may coelute with the analytes, it may lead to low assay specificity, strong the endogenous interference and high chromatogram

baseline. To solve these problems, two strategies can be adopted: 1) improve the separation and concentration process of sample processing process to make the samples entering the mass spectrum cleaner; 2) select columns with smaller filler particle size or longer column length to achieve better chromatographic separation and extend the separation time to eliminate interference as much as possible.

Can there be a new mass spectrometry solution to the challenge of analyzing these compounds? Differential Mobility Spectrometry (DMS) is a new separation technique which was commercially available in 2007 [18]. The DMS instrument is basically a drift cell consist of two parallel flat plates which placed in the ionization source before quadrupole 0. It can be installed or removed in minutes with no need to break vacuum or use any tools. As the ions migrate towards the walls of the DMS cell at different rates, they will be separated. When a RF voltage (Separation Voltage, SV) applied across the plates, a second voltage offset (Compensation Voltage, CoV) of the target ions can be corrected along the axis of the DMS cell and towards the orifice. Other species with incorrect CoV will migrate away from the straight line due to the difference in mobility. Consequently, only the target ions can enter the MS when other ions cannot reach the exit of the differential mobility cell.

Separation in DMS is based on the difference of drift times in the electrical field, which is related to the size, shape, charge state, and chemical interactions of the compounds [18]. It provides an additional dimension of selectivity which increases analytical separation power, even if ions have identical m/z and chromatographic retention times. As a post-ionization technique, DMS might have an advantage over other analytic techniques on overcoming co-eluting matrix interferences and reducing background noise, especially can improve data quality in some complex biological matrices.

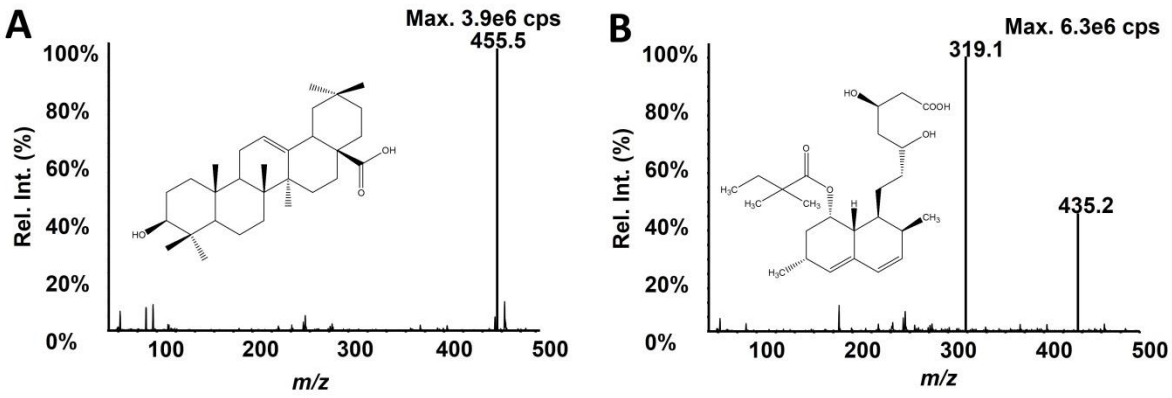
Here, we reported a successful application of modifier-assisted LC-DMS-MIM method to determine OA in rat plasma, promote a new strategy for the *in vivo* quantitation of natural compounds with poor CID efficiency or too much fragmentation.

MATERIALS AND METHODS

Reagents and chemicals

OA ($C_{30H48O3}$, CAS 508-02-1, purity >98.0%) and the ammonium salt of simvastatin acid ($C_{25H43NO6}$, CAS 139893-43-9, purity>99.0%) for use as Internal Standard (IS) were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, PR China). The structures of OA and IS are shown in Figure 1. Acetonitrile (HPLC-grade) was purchased from Fisher Scientific (Fair Lawn, NJ, USA). Distilled water was prepared from demineralized water. Solid Phase Extraction (SPE) cartridges were purchased from Waters (USA) for Oasis HLB.

Figure 1. Structures of OA and IS.



Standards and Quality Control (QC) solutions

Stock solutions of OA (100 µg/mL) were diluted with acetonitrile (90%) to produce series of standard solutions (3, 5, 10, 30, 50, 100 and 300 ng/mL) and QC solutions (5, 30 and 240 ng/mL).

Sample preparation

Plasma samples (calibration standards, QC samples or test samples) 50 µL were mixed with 25 µL IS working solution and 1 mL water, centrifuged and transferred to Solid-Phase Extraction (SPE) columns previously conditioned by elution of 1 mL methanol followed by 1 mL water SPE columns were washed with 1 mL water twice after which analytes and IS were eluted with 1 mL isopropanol: water (90:10; v/v). 50 µL eluant was mixed with 1 mL water, and finally a volume of 10 µL prepared samples was injected into the UPLC–DMS-MS/MS system.

UPLC and DMS-MS/MS conditions

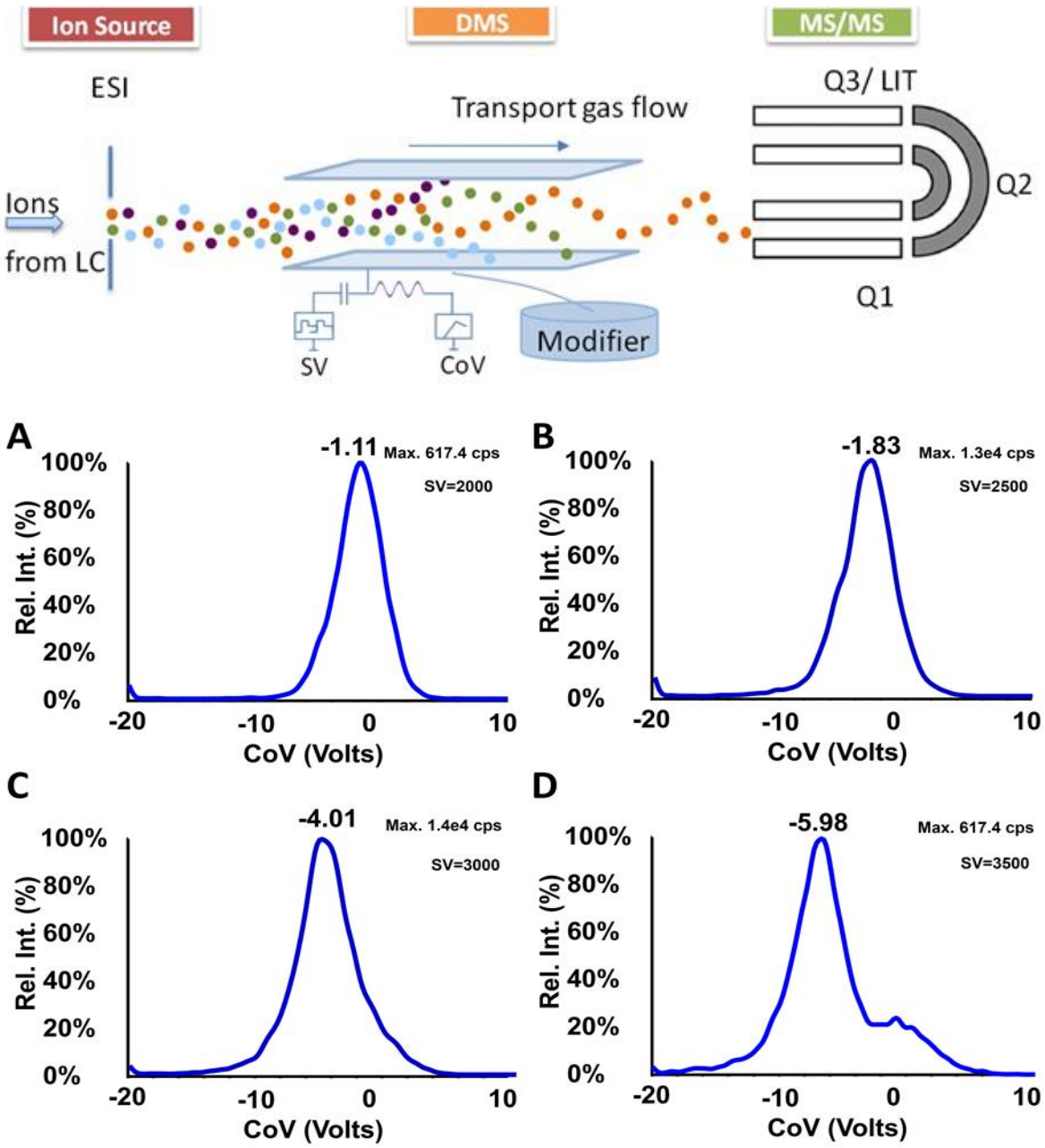
UPLC was carried out on an H class Ultrahigh Performance LC system (Perkin Elmer, Waldbronn, Germany). A C18 guard column (Phenomenex, Utrecht, Netherlands) was positioned to help minimize peak dispersion and prevent contaminants and microparticulates entering the MS. The column temperature was maintained at 40°C. The mobile phase consisted of solvent A (0.35% formic acid) and solvent B (acetonitrile) delivered at 0.5 mL/min according to the following linear gradient: 0-3 min, 90% B.

DMS-MS/MS system was from AB Sciex. A 6500 QTRAP equipped with a Selexlon® DMS cell (Concord, Ontario, Canada) mounted after the ionization source. Electro Spray Ionization (ESI) in the negative ion mode was performed by the Turbo-V ESI source set at -4500 V and 500°C. Nitrogen was used as nebulizer gas (40 units), heater gas (40 units), curtain gas (30 units) and collision gas (medium). Declustering potential was -80 V for OA and -50 V for IS, while collision energy was set to -50 eV and -22 eV for OA and IS, respectively (Figure 2).

Detection of OA was by MIM of the transition at m/z 455.5→455.5, and IS was at m/z 435.2→319.1. Data acquisition and integration were controlled by Analyst Software 1.6.2 (AB Sciex, Ontario, and Canada).

The DMS cell Temperature (DT) and offset were set to 150°C and 3.00 respectively. SV of OA was 3000 V and CoV was -4 V. Isopropanol was used as organic modifier and introduced into the transport gas at a flow rate of 250 µL/min. DMS Resolution enhancement (DR) gas (nitrogen) was set at 20 psi.

Figure 2. DMS-MS/MS system and conditions.



Assay validation

Specificity was estimated by analyzing drug-free plasma samples collected from six rats with and without spiking with analytes and IS. Intra- and inter-day precision (as Relative Standard Deviation, RSD) and accuracy (as Relative Error, RE) were evaluated by analysis of six replicate LLOQ and QC samples on three separate days. Linearity in the range 3–300 ng/mL for both analytes was evaluated by linear least-squares regression with a weighting index ($1/x^2$) of calibration curves based on peak area ratios prepared in triplicate from three separate batches. Recovery was assessed by comparing peak areas of analytes and IS in six replicates of QC samples with those of post-extracted blank plasma samples spiked at corresponding concentrations. Matrix effects were determined by comparing average peak areas of analytes and IS in six replicates of post-extraction spiked samples with those of corresponding solutions at the same concentrations. Stability of OA was investigated in stock solutions at 4°C for 6 h and in QC samples under the following conditions: At room temperature for 6 h (short-term stability); at −80°C for 7 days (long-term stability); and after three freeze–thaw cycles from −80°C to room temperature (freeze–thaw stability). Stability in processed samples on storage in auto sampler vials at 4°C for 6 h was also assessed.

Pharmacokinetic study analysis

The six rats (weight 200 ± 20 g) were purchased from Animal Experimental Center of Jilin University. Animal welfare and the experimental procedures were carried out in accordance with the Guidance for the Care and Use of Laboratory Animals of the National Research Council of USA (1996) and the related ethical regulations of Jilin University. The rats were fasted for 24 h (free access to water) before experiments. Then each of them was administrated an oral dose of 100 mg/kg OA. Blood samples (50 μL) were collected into heparinized tubes before the dose and at 5 min, 15 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h, 10 h and 24 h after dosing. Immediately, plasma was separated by centrifugation at 15000 rpm for 10 min and the resulting plasma was stored at -20°C.

A Non-compartmental PK model was developed in the rat plasma. PK data were calculated using Drug and Statistics 3.0 (DAS, China Pharmaceutical University, Nanjing, China), including maximum Concentration in plasma (Cmax), the corresponding Time (Tmax), Area Under the plasma Concentration–time curve (AUC), terminal half-life ($t_{1/2}$) and Mean Retention Time (MRT).

RESULTS AND DISCUSSION

Optimization of UPLC conditions

In view of the fact that reversed-phase chromatography is a commonly used separation system in LC-MS/MS analysis, combined with the ionization characteristics of OA, acetonitrile was selected as the organic phase. The ionization efficiency, mass spectrum response strength and chromatographic behavior of analytes at different pH were investigated. When the concentration of formic acid is adjusted to 0.35%, the sensitivity, peak shape and retention time can be adjusted to the best.

Optimization of DMS conditions

The MIM method often challenged by a significant drawback such as high background noise, because of the CID function of MS is basically turned off to minimize the loss of the precursor ion [19]. DMS delivers a new dimension of selectivity and performance for any application requiring the isolation of challenging co-eluting contaminants and reduction of high background noise. DMS parameters were optimized by ramping CoV under different conditions, including SV, DMS Resolution (DR) enhancement and DMS Temperature (DT).

SV is amplitude of the RF waveform, which alters an ion's differential mobility and thus its trajectory. When the SV changed, a new CoV is required to correct its trajectory. In general, a higher SV benefit the separation. But the value of more than 3800 V is not recommended. Because too high voltage applied to the narrow slit between electrodes will result in partial discharge. CoV is a measurement of an ion's differential mobility under specific experimental conditions. It is a DC potential used to counteract an ion's migration toward an electrode in response to the SV. Its magnitude is proportional to the ion's differential mobility. In this experiment, SV and CoV of OA were 3000 V and -4 V, respectively. DR is the pressure of gas flowing in opposition to the transport gas (nitrogen). There are five DR enhancement modes in the Sciex DMS system, including open, off, low, medium, and high. Usually, a higher transport gas flow rate will reduce the intensity but increase the power of the separation of isobaric species. Here low DR enhancement, 20 psi, was applied. It has been proved that DT had little influence on separation. Here, the value of 150°C was found to be acceptable.

It has been reported that the introduction of chemical modifier adds a new dimension to improve the resolving power and peak capacity [19]. Multiple chemical reagents can be introduced into the transport gas flow which modifies the ion interaction with the curtain gas in the DMS drift cell. Different species will have different affinities to form clusters with these organic modifiers. When the clustered ions migrating between the high field and low field portions of the applied RF, they will have difference in the rates of clustering and de-clustering. Ions will de-cluster due to the higher energy in the high field. In the contrast, the cluster will form again in the low field portion. This interaction can dramatically raise the separation capacity of the DMS cell.

Results showed that isopropanol could improve the effect of noise reduction. With the introduction of more modifiers, more clusters will be formed, and the greater change of the original separation behavior happened. But too much modifier will decrease the concentration of analytes into MS, so that bring down the intensity. Here, isopropanol was introduced to the transport gas at 250 µL/min.

Optimization of MS/MS conditions

In a triple quadrupole mass spectrometer, CID, some called Collisionally Activated Dissociation (CAD), occurs in Q2. In the collision cell, ions collide with neutral gas, which will lead to bond breakage and the fragmentation of the ions into smaller fragments. OA, as a pentacyclic triterpenoid saponin, can break

down RDA (Retro Diels -Alder reaction) in DMS by hard ionization mass spectrometry, producing two main fragment ions, one is the fragment with A and B rings as the skeleton (*m/z*=208), the other is the fragment with D and E rings as the skeleton (*m/z*=248). In soft ionization mass spectrometry, the special structure of pentacyclic triterpenoids cannot be broken to form stable fragment ions with the increase of collision energy. Therefore, it can only be detected by MIM mode, that is, the ions detected by Q1 and Q3 are the same? When MIM mode is used, a certain amount of collision energy can be added to the collision pool Q2 to reduce and eliminate some background interference. Considering that the analyte and internal standard both contain an ionizable carboxylic acid group, and do not contain heteroatoms such as N, S, etc., and the carboxyl group can ionize and dehydrogenate to generate excimer ion peak $[M-H]^-$ in the negative ion detection mode, the negative ion detection mode is selected.

After determining the detected ion reaction, we further optimized the mass spectrum parameters. During the optimization process, it was found that the value of declustering voltage had a great influence on the signal of OA: with the increase of declustering voltage, the signal response of OA gradually decreased, which may be caused by low CID efficiency and lack of stable fragments. Finally, the declustering voltage of OA was selected as-50V of OA, and other parameters were adjusted at the same time. The results showed that the ion response to *m/z* 455.5→455.5 was the best.

Assay validation

The assay was linear in the range 3-300 ng/mL with typical regression equations of y=0.0086x-0.0122 (r^2=0.9969), y=0.0088x-0.0090 (r^2=0.9953), and y=0.0093x-0.0111 (r^2=0.9953) for OA in three different days. The LLOQ was 3 ng/mL. Intra- and inter-day precision and accuracy of OA in rat plasma on assay of six replicate QC samples on three different days were satisfactory (Table 1). OA in rat plasma was stable at –80°C for 7 days, at room temperature for 6 hours, and after 3 freeze/thaw cycles. The processed samples are stable at 4°C for 6 h (Table 2).

Table 1. Accuracy and precision for the determination of oleanolic acid in rat plasma (data are based on assay of six replicate QC samples on three different days).

Spiked conc. (ng/mL)	Calculated conc. (ng/mL) (mean ± SD)	Intra-day RSD (%)	Inter-day RSD (%)	Accuracy RE (%)
3 (LLOQ)	3.04 ± 0.20	9.9	6.01	1.46
5	4.90 ± 0.43	8.29	8.79	-2.07
30	31.6 ± 1.59	3.63	5.19	5.44
240	237 ± 9.76	2.62	4.25	-0.53

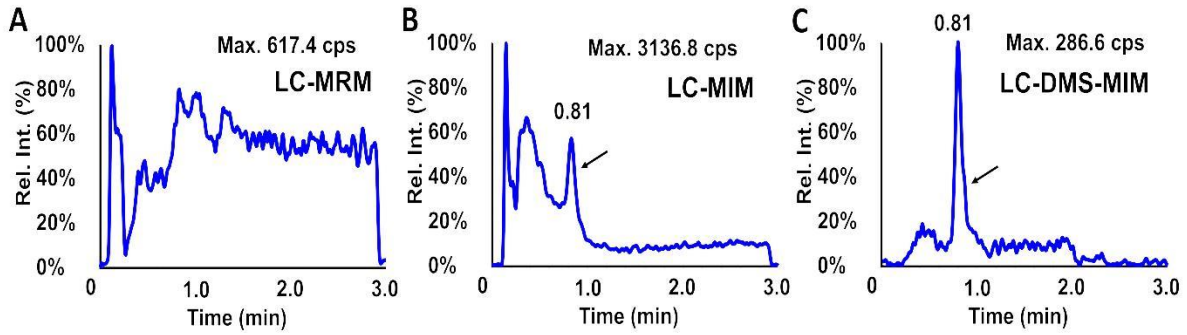
Table 2. Stability of oleanolic acid in rat plasma and prepared samples under different storage conditions.

Spiked conc. (ng/mL)	Storage at –80°C for 7 days		Storage at room temperature for 6 hours		Freeze/thaw stability (3 cycles)		Processed samples stability (4°C for 6 h)	
	Mean ± SD	RE (%)	Mean ± SD	RE (%)	Mean ± SD	RE (%)	Mean ± SD	RE (%)
5	4.86 ± 0.21	-2.8	4.74 ± 0.41	-5.2	4.75 ± 0.25	-4.93	4.76 ± 0.32	-4.8
30	28.8 ± 1.49	-4	27.5 ± 0.93	-8.22	28.8 ± 2.05	-3.89	28.8 ± 2,69	-4
240	216 ± 5.69	-9.86	222 ± 3.79	-7.64	220 ± 6.43	-8.47	225 ± 8.14	-6.11

LC-DMS-MIM compared with LC-MRM and LC-MIM

Initially, LC-MRM method and LC-MIM method were compared with LC-DMS-MIM method. The chromatograms show that in LC-MRM, OA was almost submerged in the high background noise (Figure 3A) and in LC-MIM it co-eluted with the interference (Figure 3B). In contrast, it showed much more improved in specificity and signal-to-noise ratio when using LC-DMS-MIM (Figure 3C), indicating that DMS could decrease interference and background noise significantly.

Figure 3. LC-MRM, LC-MIM and LC-DMS-MIM methods.



Pharmacokinetic study of OA using UPLC-DMS-MIM

Mean plasma concentration–time curves for OA and calenduloside E in rats are shown in Figure 4 with corresponding PK parameters listed in Table 3. After oral administration of OA, the mean C_{max} of OA in plasma was only 78.07 ng/L, indicating that OA may be transformed into other metabolites. After monitoring the glucuronic acid binding compound of OA, calenduloside E, it was found that the AUC of calenduloside E was about 50 times of OA and the C_{max} is about 30 times of OA. The plasma concentration

time curve of OA showed two absorption peaks, the first peak was at 60 min, the second peak was at 360 min. This may be due to the absorption of OA in the enterohepatic circulation, which is then transported to the liver, where it is converted into the glucuronide binding compound calenduloside E, which was excreted in bile later, and transported to ileum again, finally hydrolyzed to OA.

Figure 4. Mean plasma concentration–time curves for OA and calenduloside E in rats. Note: ( ) Oleanolic acid; ( ) Calenduloside E

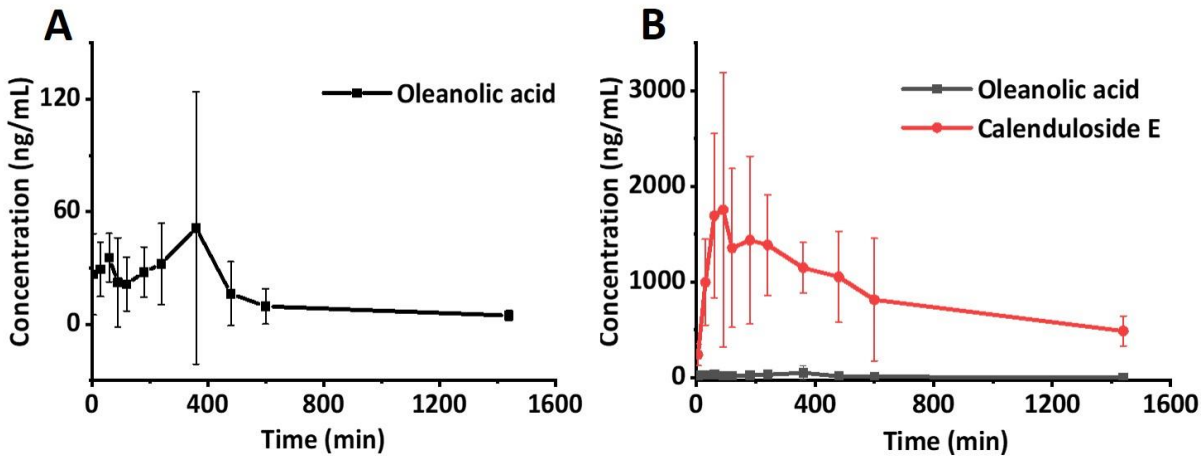


Table 3. Pharmacokinetic parameters of oleanolic acid and calenduloside E in rats after oral administration of 100 mg kg/mL oleanolic acid (data are mean ± S.D. n=6).

Parameter	Unit	Mean ± SD for OA	Mean ± SD for CE
AUC_{0-t}	ng*h/L	386.14 ± 148.80	21025.51 ± 5779.20
$AUC_{0-\infty}$	ng*h/L	413.06 ± 155.03	43322.62 ± 35801.41
MRT_{0-t}	h	7.34 ± 1.66	9.32 ± 1.82
$MRT_{0-\infty}$	h	9.21 ± 2.64	22.19 ± 10.85
$t_{1/2}$	h	5.58 ± 3.24	11.96 ± 7.08
T_{max}	h	4.25 ± 2.44	3.92 ± 3.58
C_{max}	ng/L	78.07 ± 59.25	2231.67 ± 1219.83

CONCLUSION

The problems of low ionization efficiency or strong interference in the analysis of compounds *in vivo* can only be solved through complex sample processing and longer analysis time, and some may not be solved. We took OA as an example to show that LC-DMS-MIM has special advantages may tackle some thorny problems which cannot be solved with LC-MRM or LC-MIM, for instance, pentacyclic triterpenoid-like

molecules. We chose the most abundant ion for both quadrupole 1 and quadrupole 3 to achieve good sensitivity. An organic modifier, isopropanol, was facilitated a good combination of separation and sensitivity. Comparing with MIM or MRM, DMS-MIM can reduce the matrix interference and chemical background. Therefore, DMS may become a novel analysis instrument coupled with chromatography and mass spectrometry. Recently, LC-DMS-MIM has not been widely used in the bioanalysis area yet, this report provided support data for a wider scope of MS application on determination of analytes with poor CID efficiency or too much fragmentation in biological matrices.

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REFERENCES

1. Ayeleso T B, et al. Oleanolic Acid and Its Derivatives: Biological Activities and Therapeutic Potential in Chronic Diseases. Molecules. 2017; 22:1915.
2. Edathara P M, et al. Inhibitory role of oleanolic acid and esculetin in HeLa cells involve multiple signaling pathways. Gene. 2021; 771:145370.
3. Baer-Dubowska W, et al. Anti-Cancer Potential of Synthetic Oleanolic Acid Derivatives and Their Conjugates with NSAIDs. Molecules. 2021; 26:4957.
4. Tang Z Y, et al. Anticancer activity of oleanolic acid and its derivatives: Recent advances in evidence, target profiling and mechanisms of action. Biomed Pharmacother. 2022; 145:112397.
5. Xu Y, et al. High-throughput metabolomics investigates anti-osteoporosis activity of oleanolic acid via regulating metabolic networks using ultra-performance liquid chromatography coupled with mass spectrometry. Phytomedicine. 2018; 51:68-76.
6. Shi Y, et al. Research Advances in Protective Effects of Ursolic Acid and Oleanolic Acid Against Gastrointestinal Diseases. Am J Chin Med. 2021; 49:413-35.
7. Djeziri F Z, et al. Oleanolic acid improves diet-induced obesity by modulating fat preference and inflammation in mice. Biochimie. 2018; 152:110-120.
8. Luo H Q, et al. Lipid-lowering effects of oleanolic acid in hyperlipidemic patients. Chin J Nat Med. 2018; 16:339-346.
9. Zhang X, et al. Advances on the Anti-Inflammatory Activity of Oleanolic Acid and Derivatives. Mini Rev Med Chem. 2021; 21:2020-2038.
10. Tsao S M, et al. Retraction of "Antioxidative and Antiinflammatory Activities of Asiatic Acid, Glycyrrhizic Acid, and Oleanolic Acid in Human Bronchial Epithelial Cells". J Agric Food Chem. 2017; 65:3251.

11. Xin C, et al. The novel nanocomplexes containing deoxycholic acid-grafted chitosan and oleanolic acid displays the hepatoprotective effect against CCl(4)-induced liver injury *in vivo*. Int J Biol Macromol. 2021; 185:338-349.
12. Loos G, et al. Quantitative mass spectrometry methods for pharmaceutical analysis. Phil. Trans. R. Soc. A. 2016;374.
13. Zheng Z, et al. Simultaneous Determination of Oleanolic Acid and Ursolic Acid by *in vivo* Microdialysis via UHPLC-MS/MS Using Magnetic Dispersive Solid Phase Extraction Coupling with Microwave-Assisted Derivatization and Its Application to a Pharmacokinetic Study of ArctiumIappa L. Root Extract in Rats. J. Agric. Food Chem. 2018; 66:3975-3982.
14. Kim E, et al. Simultaneous determination of 3-O-acetyloleanolic acid and oleanolic acid in rat plasma using liquid chromatography coupled to tandem mass spectrometry. J Pharm Biomed Anal. 2016; 118:96-100.
15. Zhao L, et al. Simultaneous Determination of Oleanolic and Ursolic Acids in Rat Plasma by HPLC-MS: Application to a Pharmacokinetic Study After Oral Administration of Different Combinations of QingGanSanJie Decoction Extracts. J Chromatogr Sci, 2015; 53:1185-1192.
16. Shi M, et al. Pharmacokinetic study of calenduloside E and its active metabolite oleanolic acid in beagle dog using liquid chromatography–tandem mass spectrometry . J Chromatogr B Analyt Technol Biomed Life Sci. 2014; 951-952:129-134.
17. Xia Y Q, et al. Differential mobility spectrometry combined with multiple ion monitoring for bioanalysis of disulfide-bonded peptides with inefficient collision-induced dissociation fragmentation. Bioanalysis. 2017; 9:183-192.
18. Schneider B B, et al. Differential mobility spectrometry/mass spectrometry history, theory, design optimization, simulations, and applications. Mass Spectrom Rev. 2016; 35:687-737.
19. Schneider B B, et al. Chemical effects in the separation process of a differential mobility/mass spectrometer system. Anal Chem, 2010; 82:1867-1880.