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A Selective Method for the Quantitation of Allopurinol in Human Plasma Using Differential Ion Mobility Spectrometry

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Overview

PURPOSE

The aim of the present research was to leverage the capabilities of SelexION+ differential mobility spectrometry (DMS) to discriminate allopurinol from the isobaric endogenous interference, hypoxanthine (Figure 1), present in both human plasma and blood.

METHOD

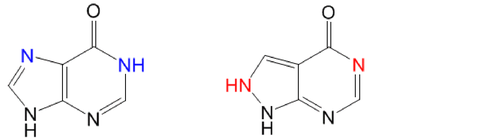
Allopurinol was fortified in human plasma and hemolyzed plasma (5%) at concentrations of 20–5000 ng/mL and extracted by protein precipitation using ACN. Allopurinol-^{13}C-$^{15}N_2$ was used as the internal standard. Extracts were analyzed by both LC-MRM and LC-DMS-MRM.

RESULTS

An LC-DMS-MRM method was developed using ACN:IPA (3:1) modifier to differentiate allopurinol from hypoxanthine, whose concentration was approximately 100-fold greater in hemolyzed plasma (Figure 3) than the allopurinol LOQ.

INTRODUCTION

Allopurinol is a xanthine oxidase inhibitor preventing the conversion of hypoxanthine and xanthine to uric acid, and is therefore used in the treatment of gout and renal calculi. The development of an LC-MS/MS assay for allopurinol in human plasma was challenged by the endogenous presence of the isobaric compound hypoxanthine. However, when coupling the SelexION+ DMS to LC-MS/MS, allopurinol could be separated from hypoxanthine, based on differences in their ion mobility in the presence of dopant. Under the optimized conditions of the DMS described herein, a selective and sensitive method could be developed demonstrating precision and accuracy data sufficient to meet all acceptance criteria for a validated method (Table 2).



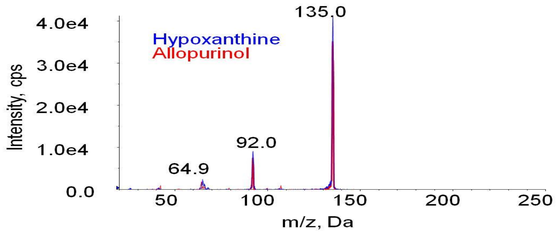


Figure 1. Structures and MS/MS fragmentation of allopurinol and hypoxanthine

Method

SAMPLE PROCESSING

- 100 µL of plasma was fortified with Allopurinol-^{13}C-$^{15}N_2$ and precipitated with 300 µL of ACN
- Samples were vortexed, centrifuged, and further diluted for LC-MRM analysis

CHROMATOGRAPHY

- Hypercarb (50 x 2.1 mm id, 5 µm)
- Gradient elution with 0.1% acetic acid in water and ACN

MASS SPECTROMETRY

- SCIEX Triple Quad 6500+ operated in (+)ESI-MRM mode with SelexION+ DMS (Figure 2)
- MRM transitions:
  - Allopurinol and hypoxanthine: *m/z* 135.0 > 92.1
  - Allopurinol-^{13}C-$^{15}N_2$: *m/z* 138.0 > 95.1

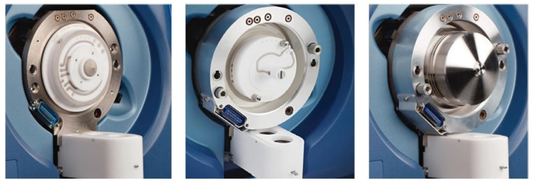


Figure 2. SelexION+ interfaced to SCIEX 6500+ Triple Quad

RESULTS

The use of methanol, acetonitrile, isopropyl alcohol or acetone as the DMS dopant demonstrated little to no separation of allopurinol from hypoxanthine (Figure 4), whilst 50:50 ACN:IPA or 75:25 ACN:IPA allowed the resolution of the isobars at a low DMS temperature and a high modifier flow rate (Figure 5). However, due to the partial overlap of the allopurinol and hypoxanthine ionogram profiles, it was necessary to offset the compensation voltage for allopurinol by +4 V (Figure 6), in order to completely eliminate the hypoxanthine response (Table 1 and Figure 7).

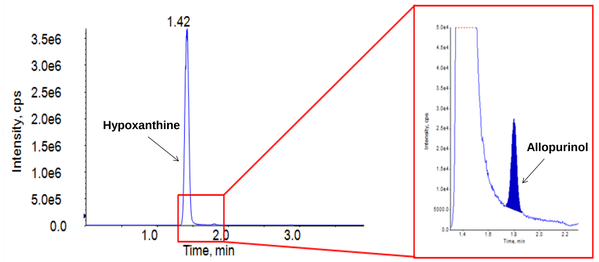


Figure 3. LC-MRM profile of allopurinol in 5% hemolyzed plasma

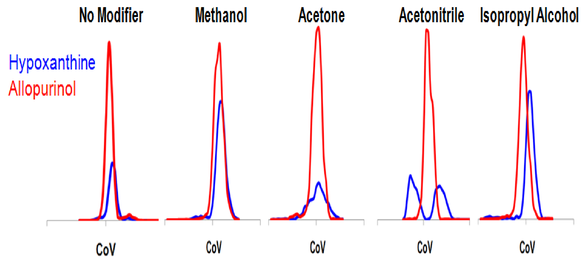


Figure 4. CoV profiles as a function of dopant at separation voltage (SV) 3.8 kV

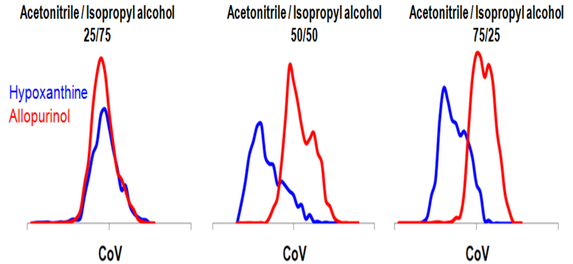


Figure 5. CoV profiles of dual-component modifiers

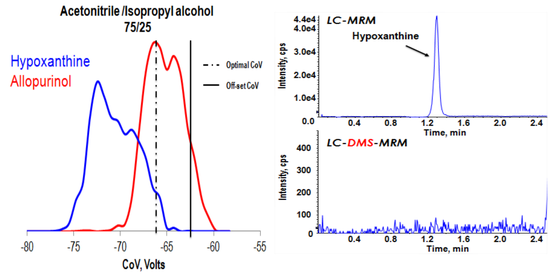


Figure 6. Optimal CoV offset for discrimination of allopurinol from hypoxanthine

Table 1. Assay specificity comparison of LC-MRM vs. LC-DMS-MRM

Donor	Hypoxanthine Conc. (ng/mL)	
	MRM	DMS-MRM
LS8830161	111.0	No peak
LS2386554	283.6	No peak
LS8826953	300.5	No peak
LS2387399	175.0	No peak
LS8829868	195.9	No peak
BRH1316305	110.9	No peak
BRH1288641	39.8	No peak
BRH1316291	124.9	No peak
BRH1348569 (Lipemic Plasma)	350.0	No peak
LS8830161 (Hemolyzed Plasma)	***1733.3***	***No peak***

Table 2. Within-run precision and accuracy (n = 6)

	LOQ QC 20.0 ng/mL	Low QC 60.0 ng/mL	Mid QC 800.0 ng/mL	High QC 3750.0 ng/mL
% Nominal	98.9	93.4	92.8	96.9
% CV	5.5	6.2	1.6	5.7

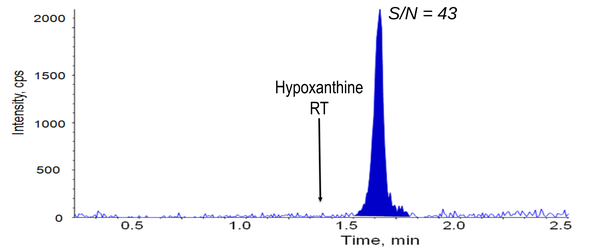


Figure 7. Allopurinol LOQ chromatogram using LC-DMS-MRM

Conclusions

Through the implementation of the SelexION DMS and the optimization of the chemical modifier, the differentiation of allopurinol from interfering hypoxanthine was achieved, thereby allowing specificity criteria to be met whilst improving the assay LOQ. In addition, complex chromatographic separations were avoided, allowing for a high throughput assay .