Evaluation of the Analytical Characteristic of a Differential Mobility Analyzer coupled to a Triple Quadrupole System (DMA-MSMS)

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Hassan Javaheri [1]; Yves Le Blanc[1]; Bruce A. Thomson[1]; Juan Fernandez de la Mora[2]; Juan Rus[3]; Juan Antonio Sillero Sepúlveda[3] – [1]MDS Analytical Technologies, Concord, ON Canada; [2]Yale University, Hew Haven, CT; [3]SEADM, Valladolid, Spain.

ABSTRACT

A Differential Mobility Analyzer has been installed on an API 3000 to provide mobility separation of ions prior to MS/MS analysis. A variety of samples have been tested to show the resolving power of the DMA, and to demonstrate the improved signal-to-noise provided by this type of DMA/MS/MS in an MRM mode of operation.

INTRODUCTION

Differential mobility analysis (DMA) is a technique that enables separation of species in space based on their difference in ion mobility[1-3]. Since the conformation of a molecule can have a significant effect on its mobility, this technique could assist in eliminating isobaric interferences in MS and MSMS analysis. To evaluate this capability, a DMA device was installed between the ion source and the orifice of an MS system. The DMA-MSMS combination was evaluated in terms of ion transmission performance as well as in terms of resolution of isobaric species through infusion experiments. Further test were performed on samples spiked in a biological matrix to asses the improvement of S/N when the technique is coupled to LC and MSMS.

MATERIALS AND METHODS

The DMA device tested in this work was built by SEADM (Sociedad Europea de Analisis Diferencial de Movilidad SL, Madrid, Spain) and initially tested on an API 365 at Yale University. It was operated at a sheath gas flow rate where conditions remained laminar through the working separation region. Sensitivity and resolution were characterized as a function of the DMA parameters (gas flow and voltage). The DMA was installed at MDS Analytical Technologies on a modified API 3000® LC-MS/MS System and coupled to an LC system through a TurboIonSpray® source.

All chemicals were obtained from Sigma (St Louis, MO) or from Cambridge Isotope (Andover, MA) and used without purification. Urine samples were obtained from internal volunteers and typically diluted 1:1 or 1:5 prior before being spiked with analytes of interest. No additional sample preparation was performed prior to LC separation.

Chromatography was performed using a Luna C18(2) column (2x50mm, 5u) from Phenomenex (Torrance, CA) with gradient elution. Samples were injected using Perkin Elmer series 200 autosampler and micro pumps. For MRM acquisition, optimal DMA voltage and collision energy (CE) conditions were used for all fragment ions monitored..

RESULTS

The DMA has been designed to provide a very low level of turbulence in the region between the slit (ion sampling from source) and the orifice (ion sampling into the MS vacuum). The design of the DMA ensures that laminar flow can be maintained thought the chamber even at Reynolds numbers well beyond the usual threshold for turbulent transition. Molecular ions or clusters from the ion spray enter through a slit into the sheath gas region. The second plate is provided with an outlet orifice that is located approximately 20 mm downstream of the inlet slit. A potential difference is applied between the first and second plate which causes the flow of ions, introduced via the inlet slit, to migrate towards the outlet orifice. The laminar gas flow displaces ion flight trajectories along the flow axis; so that only the ions with mobility near a specific value reach the second plate close enough to the outlet orifice to be sampled through it.
The particles move downstream at the speed of the gas flow, so that particles with low mobility are carried further downstream and particles with higher mobility cross the chamber quickly. The transit time for ions through the DMA is approximately 0.33 msec
With this DMA resolution of 70 was achieved

Figure 1- Differential mobility analysis (DMA) Principle of Operation.

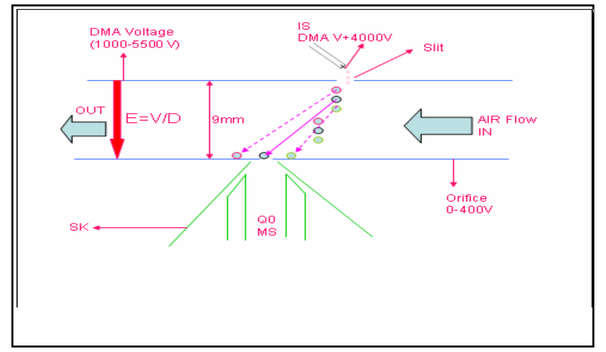


Figure 2. DMA/MS interface

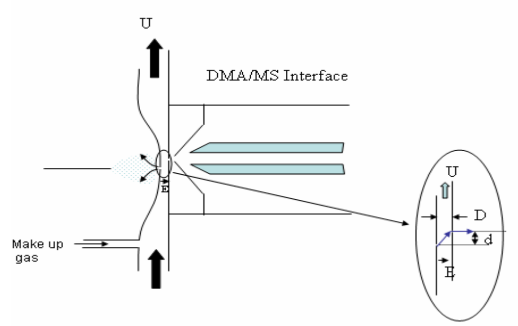
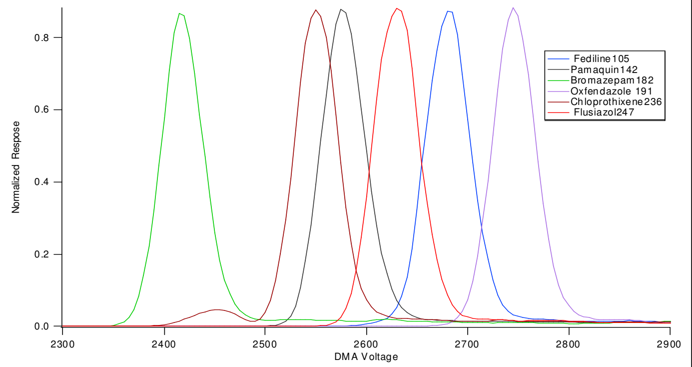


Table 1. DMA operational parameter

Sheath gas velocity (m/sec)	60
Sheath gas flow (L/sec)	10
Ion transit time (m sec)	0.4
Resolution (dV/ V)	70

Figure 3.Resolution- 6 isobaric drugs (M+H)+ = 316



Oxycodone and oxfendazole are isobaric species at m/z 316. Though specific fragment ions can be used to selectively detect each species, there is a common fragment (MRM 316-159 @ 45eV) for both species that could be used to mimic the presence of interference in controlled fashion. This approach was used to asses the effectiveness of the DMA at reducing interferences. Figure 4 shows the DMA optimization between oxycodone and oxfendazole when operated with the TurboIonspray at 450ºC (conditions for LC at 450uL/min). The optimum value was used for each compound. Figure 5a shows the LC-MSMS separation without the DMA device, showing that each compound interferes in the analysis of the other. However, when the acquisition is performed at the DMA voltage specific to oxycodone (Fig.5B) and oxfendazol (Fig.5C), the respective interferences are reduced significantly (>17x). Further reduction could be obtained if the DMA voltage were set to eliminate interference completely, with a small reduction for both analyte intensity.

Oxfendazol was then spiked at low concentration level in diluted urine (1:1) and the MRM ratio of the 3 major fragments were measured with and without the DMA system. Figure 6 shows the interference observed in 2 of the MRM transition at low level in urine. Table 2 shows the MRM ratio obtained when urine is analyzed with and without DMA at low concentration level. It is clear from that table that using the DMA does eliminate interference that would otherwise contribute to improper ratio determination, as the DMA ensures that the ratio is at least within 18% of the solution ratio.

Figure 4. Optimization of DMA voltage for oxycodone and oxfendazol with TIS

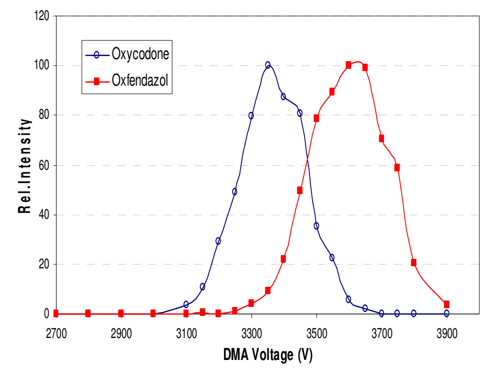
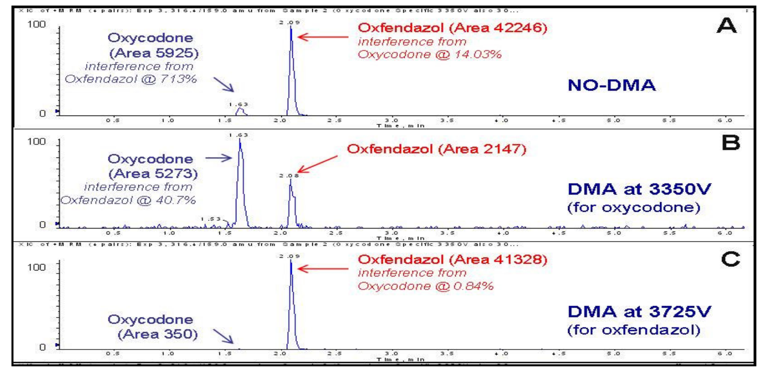


Figure 5. LC-DMA-MSMS analysis of oxycodone and oxfendazol in solution



Oxycodone 250pg/uL and Oxfendazol 500pg/uL in solution were injected and analysed by LC-MSMS (A), by LC-DMA-MSMS with DMA at 3350V (B) and by LC-DMA-MSMS with DMA at 3725V (C). The common MRM (316-159) for xycodone and oxfendazol at 45eV was used for this analysis.

Figure 6. LC-DMA-MSMS analysis of 25pg/uL of oxfendazole spiked in urine sample (dil. 1:1)

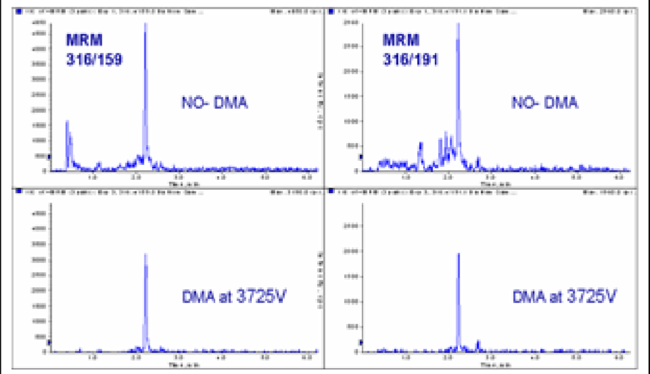


Table 2. Ratio of 3 major MRM for Oxfendazol in biological matrix at different levels (w/wo DMA)

Solution	Conc. (pg/uL)	Inst.Cond.	Peak Area for MRM 316-159	316-191	316-239	Ratio 159 to 191	Ratio 159 to 239
STD Solution	500	DMA	28401	17303	2321	1.64	12.24
		DMA	30698	17945	2291	1.71	13.40
					Average	1.68	12.82
Urine Sample 6 (1:1)	125	NO DMA	57324	32995	10028	1.74	5.72
					Dev. From average ratio>>>	*3.7*	*-55.4*
Urine Sample 6 (1:1)	25	NO DMA	10678	8935	6816	1.20	1.57
					Dev. From average ratio>>>	*-28.7*	*-87.8*
Urine Sample 6 (1:1)	125	DMA	45515	29096	3999	1.56	11.38
					Dev. From average ratio>>>	*-6.7*	*-11.2*
Urine Sample 6 (1:1)	25	DMA	7899	4714	520	1.68	15.19
					Dev. From average ratio>>>	*0.0*	*18.5*

Figure 7. DMA voltage for 54 compounds

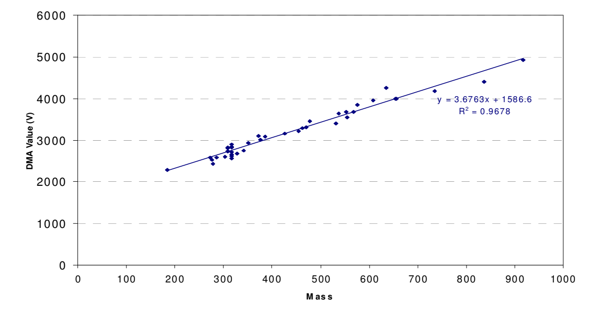


Figure 8. DMA for drug and corresponding glucuronide

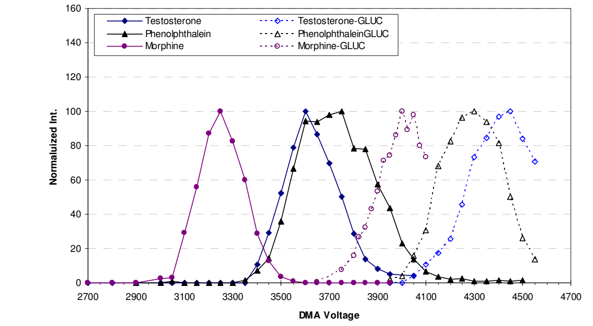


Figure 9. Analysis of Glucuronide with and without DMA to discriminate in source fragmentation.

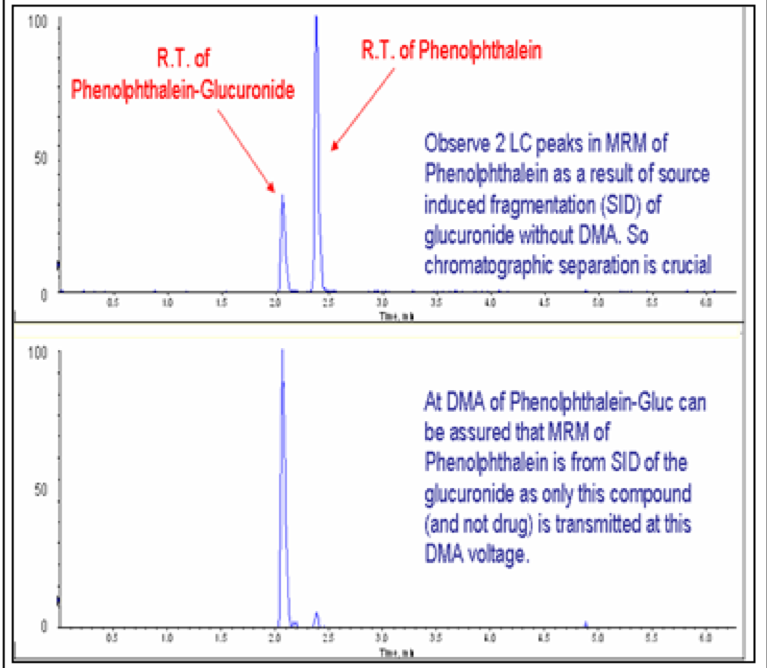


Figure 7 shows the optimum DMA voltage obtained for 54 different compound ranging from m/z 186 to 940. This shows a reasonably linear behavior between the DMA voltage and the mass of the compound, suggesting that one could at least predict regions of interest based on expected mass. This is further emphasized in Figure 8 where the DMA voltage of 3 compounds and their corresponding glucuronide metabolites. Here, once the DMA voltage of the parent compound is known, simply operating the DMA at 700V higher would ensure detection and transmission of the corresponding glucuronide. Figure 9 shows that the DMA can be used to discriminate detection of parent drug signal originating from in-source fragmentation of the glucuronide metabolite. This would enable faster analysis of both compounds without the risk of interference.

CONCLUSIONS

This work showed that a DMA interface can effectively be used to separate isobaric species which in turn can be used to further improve the selectivity of MRM. This was demonstrated for the analysis of oxfendazole in urine samples where the ratio of 3 fragment ions was sustained at low concentration. Another interesting aspect is that one could predict the DMA voltage range of interest for metabolites associated with a given compounds. The DMA could then either be used to improve selective detection of the metabolite OR ensure discrimination from source-CID fragmentation of the metabolite and the parent drug.

REFERENCES

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2. Bruce Thomson, Hassan Javaheri, John Van Nostrand, K. W. Michael Siu, Juan Fernandez de la Mora, ASMS, Cincinnati OH 2007
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