

M1530-03-019 Selective Quantitation of 1,3-Propanediol in Dog Plasma Using Differential Ion Mobility Spectrometry

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PURPOSE

Propylene glycol (PG) has numerous pharmaceutical applications such as a solubilizer, alleviant and dosing vehicle (containing up to 80% of PG). As a major constituent of PG, 1,2-propanediol (PrDL) is considered relatively non-toxic, however the toxicity of its less abundant isomer 1,3-PrDL is unknown. Consequently, there is an increased demand for the determination of 1,3-PrDL in biological matrices, however the development of an LC-MS/MS assay is complicated by the predominance of isobaric 1,2-PrDL. Therefore, an orthogonal level of selectivity using differential ion-mobility spectrometry (DMS) was implemented to separate 1,2-PrDL and 1,3-PrDL prior to MS/MS analysis, allowing high-throughput analysis by obviating the requirement for chromatographic resolution of isomers.

OBJECTIVE

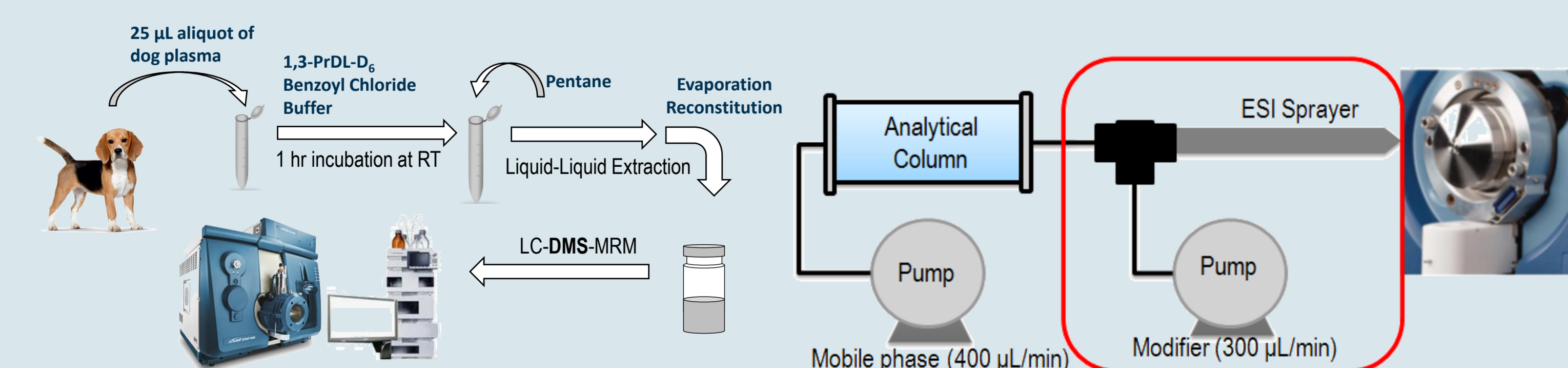
To distinguish 1,3-propanediol (1,3-PrDL) from the interfering isomer 1,2-PrDL in dog plasma using differential mobility spectrometry (DMS).

METHODS

1,3-Propanediol was fortified in dog plasma from 0.20 – 100.00 µg/mL. Samples were treated with 25 µL of internal standard (1,3-PrDL-D₆), 150 µL of buffer and 25 µL of benzoyl chloride, mixed and left to derivatize for one hour at room temperature. Following derivatization, samples were extracted into pentane and the organic phase evaporated and reconstituted (Figure 1). Chromatographic separation was achieved under isocratic conditions on a Halo C₁₈ column with 0.02% acetic acid and methanol. Parent ions were formed by positive ion electrospray and detected in MRM mode for the transitions m/z 163.1 > 105.1 (1,3-PrDL) and m/z 169.1 > 105.1 (1,3-PrDL-D₆) using a SCIEX Triple Quad 6500+ equipped with SelexION+. Methanol was infused as post-column modifier at a flow rate of 300 µL/min (Figure 2).

Figure 1. Sample preparation procedure of 1,3-PrDL in dog plasma

Figure 2. The scheme of introducing post-column modifier PrDL in dog plasma



RESULT(S)

Derivatization and In-Source Fragmentation

Benzoyl chloride (BzCl) is highly reactive towards glycols in aqueous solution under strongly alkaline conditions via the Schotten-Baumann reaction (Figure 3). The +ESI/ full scan-mass spectrum of derivatized 1,3-PrDL is presented in Figure 4 and was characterized by a base peak ion at m/z 163.1, suggesting formation of the in-source fragment ion $[M+H-C_6H_5COOH]^+$. The relatively high abundance of this fragment ion may be rationalized by the stable ring structure proposed in Figure 4.

Elimination of 1,2-Propanediol Interference via DMS

As 1,3-PrDL and 1,2-PrDL exhibit similar chromatographic retention and identical MRM transition, isomer differentiation by LC-MRM with reasonable throughput is problematic. However, differences in the gas-phase ion mobility of 1,3- and 1,2-PrDL can be exploited to separate these isomers, the efficacy of which is improved by the superimposition of alternating low and high electric fields to the DMS cell in the presence of chemical modifier. In this manner, the difference in ion mobility between isomers is enhanced due to greater disparity between physical cross-section, catalyzed by clustering with dopant at low potential and declustering at high potential. In the presence of MeOH introduced post-column (Figure 2), discrimination between isomers was achieved as outlined in the Figure 5. A combined SV of 3.8 kV with 14V CoV eliminated 1,2-PrDL in blank dog plasma extracts (Table 1), whilst increasing the S/N ratio for the LOQ response of 1,3-PrDL (Figure 6).

Method Performance

The application of LC-DMS-MRM for the determination of 1,3-PrDL from 0.20 – 100.00 µg/mL met acceptance criteria for within-run precision and accuracy (Table 2), achievable due only to the elimination of ubiquitous 1,2-PrDL.

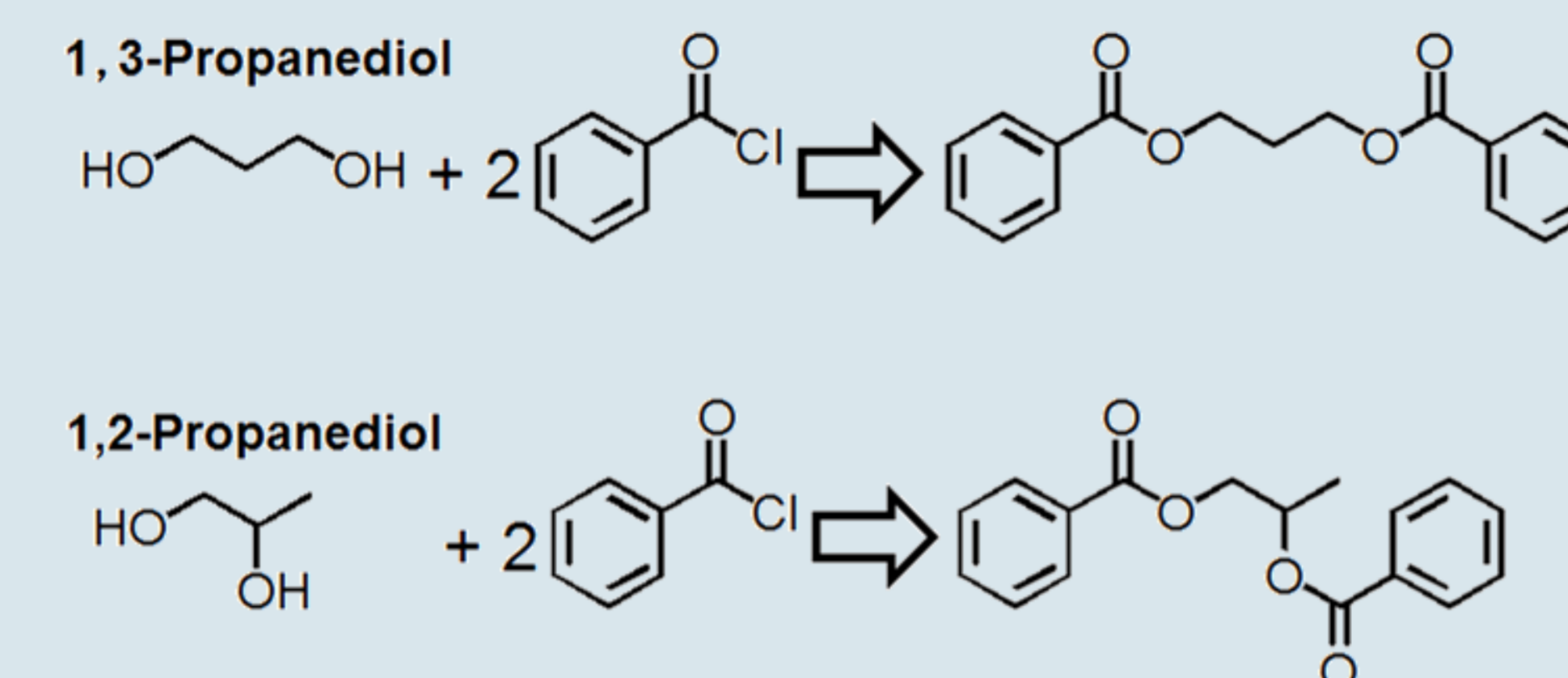


Figure 3. Derivatization of 1,3-PrDL and 1,2-PrDL with BzCl

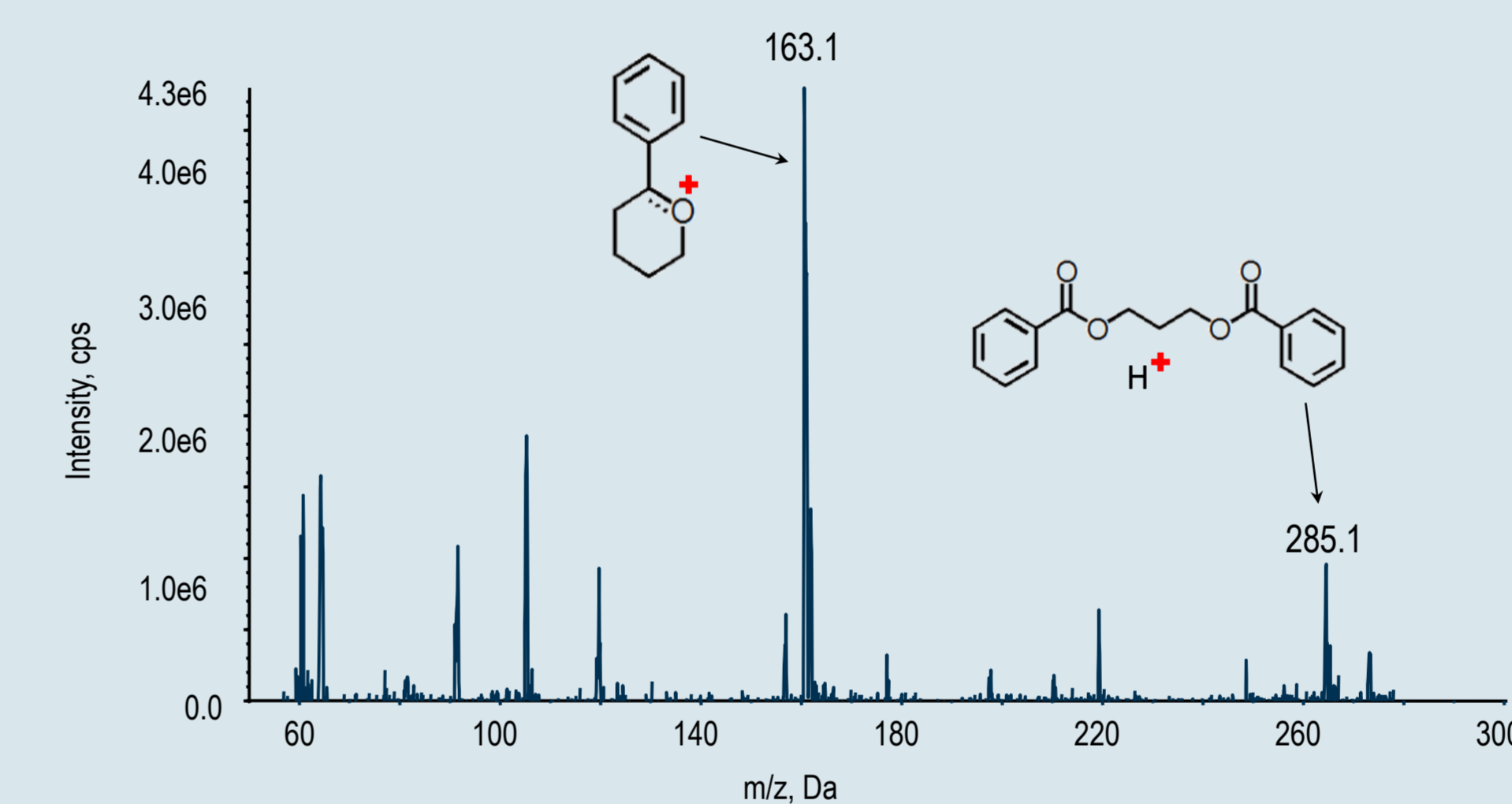


Figure 4. Mass spectrum of benzoyl-derivatized 1,3-PrDL

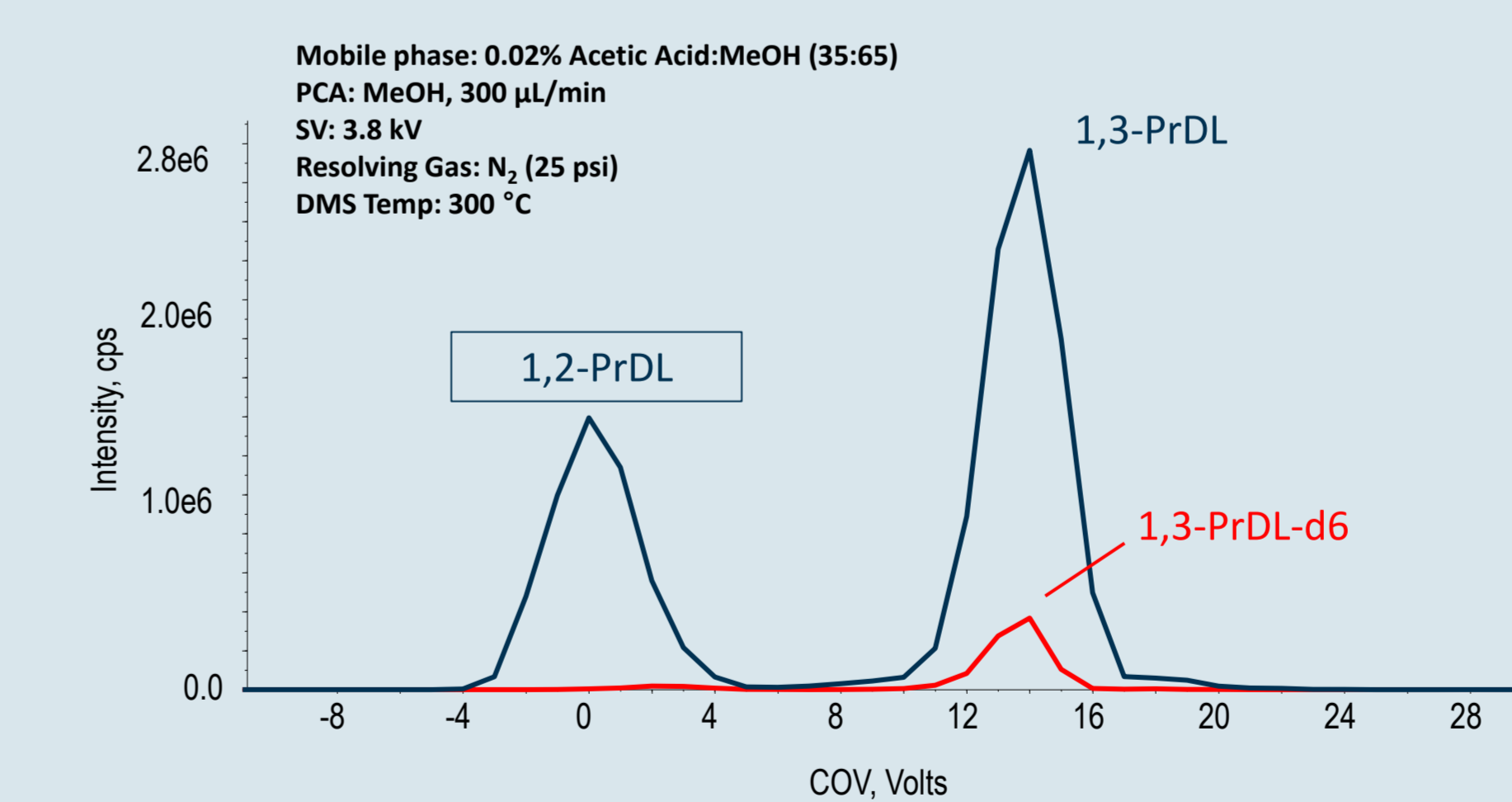


Figure 5. Ionogram of benzoylated 1,2-PrDL and 1,3-PrDL

Table 1. 1,2-PrDL in dog plasma by LC-MRM and LC-DMS-MRM

Donor	LC-MRM	LC-DMS-MRM
BGL85675	3.85 µg/mL	No peak
BGL85672	1.92 µg/mL	No peak
BGL85674	1.93 µg/mL	No peak
BGL95686 (Hemolyzed)	2.95 µg/mL	No peak

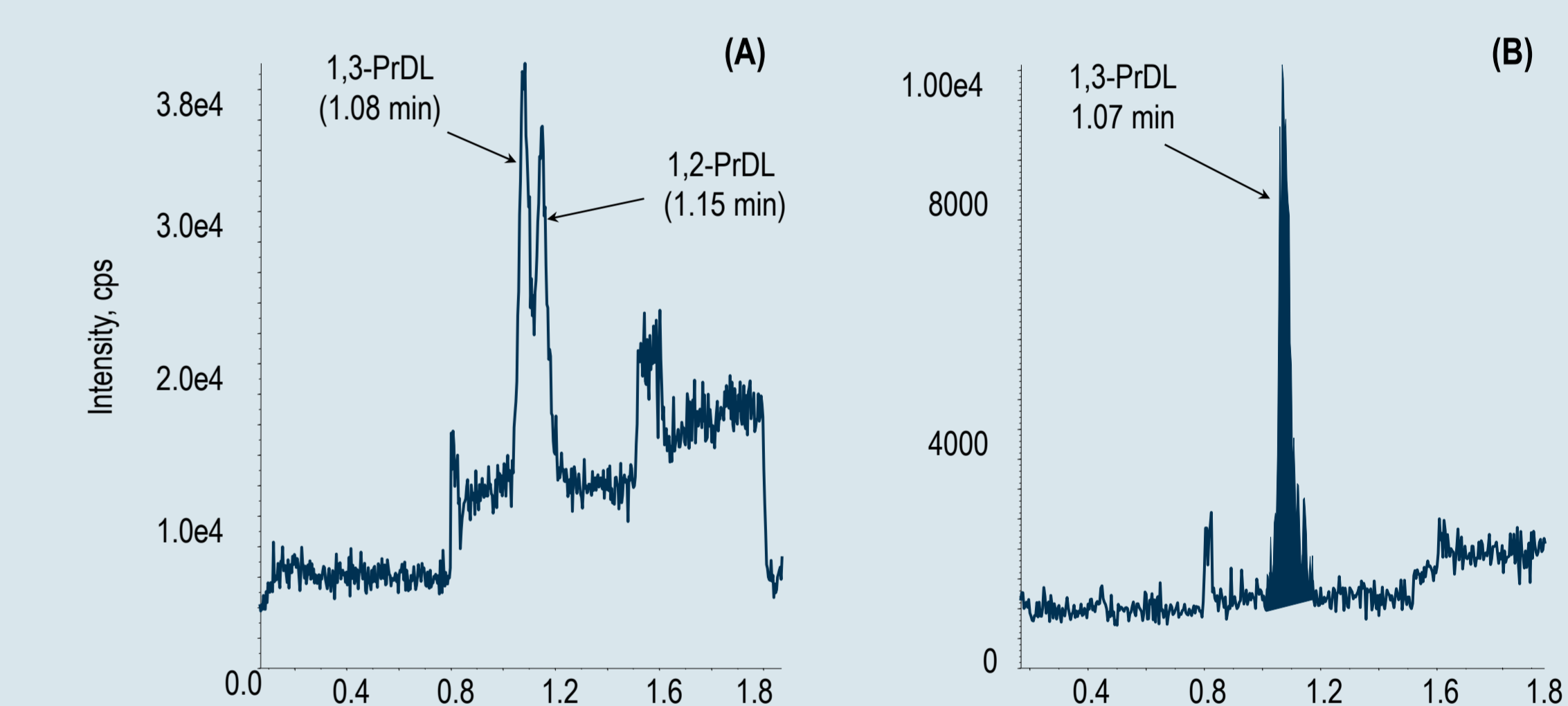


Figure 6. LLOQ response by (A) LC-MRM and (B) LC-DMS-MRM

Table 2. Within-run P&A for derivatized 1,3-PrDL in dog plasma

	QC-LOQ 0.20 µg/mL	QC-1 0.60 µg/mL	QC-2 7.00 µg/mL	QC-3 75.00 µg/mL
Statistics (n = 6)	0.21	0.63	7.22	78.03
	0.21	0.57	7.00	75.26
	0.22	0.64	7.15	75.29
	0.21	0.65	7.23	77.73
	0.22	0.65	7.50	74.51
Mean	0.22	0.63	7.21	76.01
S.D.	0.01	0.03	0.16	1.48
% C.V.	5.3	4.8	2.3	1.9
% Nominal	109.3	105.7	102.9	101.4

CONCLUSION

By leveraging an additional stage of gas-phase selectivity via DMS, the targeted LLOQ of 0.20 µg/mL was achieved for 1,3-PrDL, free of 1,2-PrDL interference. The intra-day precision was < 6% with accuracies ranging from 101 to 109% for all QCs, indicating the developed LC-DMS-MS/MS method would meet all acceptance criteria for a validatable method.



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