Highly sensitive, selective and rapid LC-MS method for simultaneous quantification of diadenosine polyphosphates in human plasma

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BACKGROUND

Diadenosine polyphosphates (ApnAs) are endogenous mediators involved in a large number of pathophysiologic processes[1,2]. In plasma, ApnAs are quantified using chromatographic and UV based methods with low selectivity due to co-eluting plasma components and requires high volume. Therefore, we developed and validated a highly sensitive, selective and rapid LC–ESI–MS method for simultaneous quantification of ApnAs (withn = 3–6) in human plasma by comparison of ESI-MS/MS fragment spectra of isolated endogenous compounds with those of pure ApnAs.

METHODS

Diadenosine polyphosphates were extracted from 100µl human plasma using weak anion exchange extraction cartridges. The separation of ApnAs was achieved using capillary C18 columns. ESI-HCT mass spectrometer (Bruker Daltonik, Germany) operated in negative ion mode was used for detection and quantification of ApnAs.

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Dilution of plasma with 100 mM ammonium formate

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Weak anion exchange SPE

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Evaporation

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Reconstitution with 1% N,N-DMHA

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LC-ESI-MS

Steps involved in preparation of plasma samples for the quantification of diadenosine polyphosphates

RESULTS

The chemical structure of ApnAs is given in Figure 1. A calibration curve was established for diadenosine polyphosphate free plasma in the concentration range of 1.9–125 nM (r2> 0.998) for all analytes (figure 4). The quantitative determination of the matrix effects revealed minor influence was quantified as −1.1 ± 2.6 for Ap3A, 0.9 ± 2.4 for Ap4A,−3.2 ± 1.7 for Ap5A and 0.3 ± 2.7 for Ap6A (% ± SEM each) (Figure 3). The intra- and inter-day accuracies were in the range of 91.4% and 110.9%. The intra- and inter-day precisions were determined as 0.1% and 11.4%, respectively (Table 1). The mean plasma concentrations of ApnAs were quantified as 31.9 ± 5.9 nM for Ap3A, 40.4 ± 6.6 nM forAp4A, 10.7 ± 1.5 nM for Ap5A and 10.0 ± 18.9 nM for Ap6A.

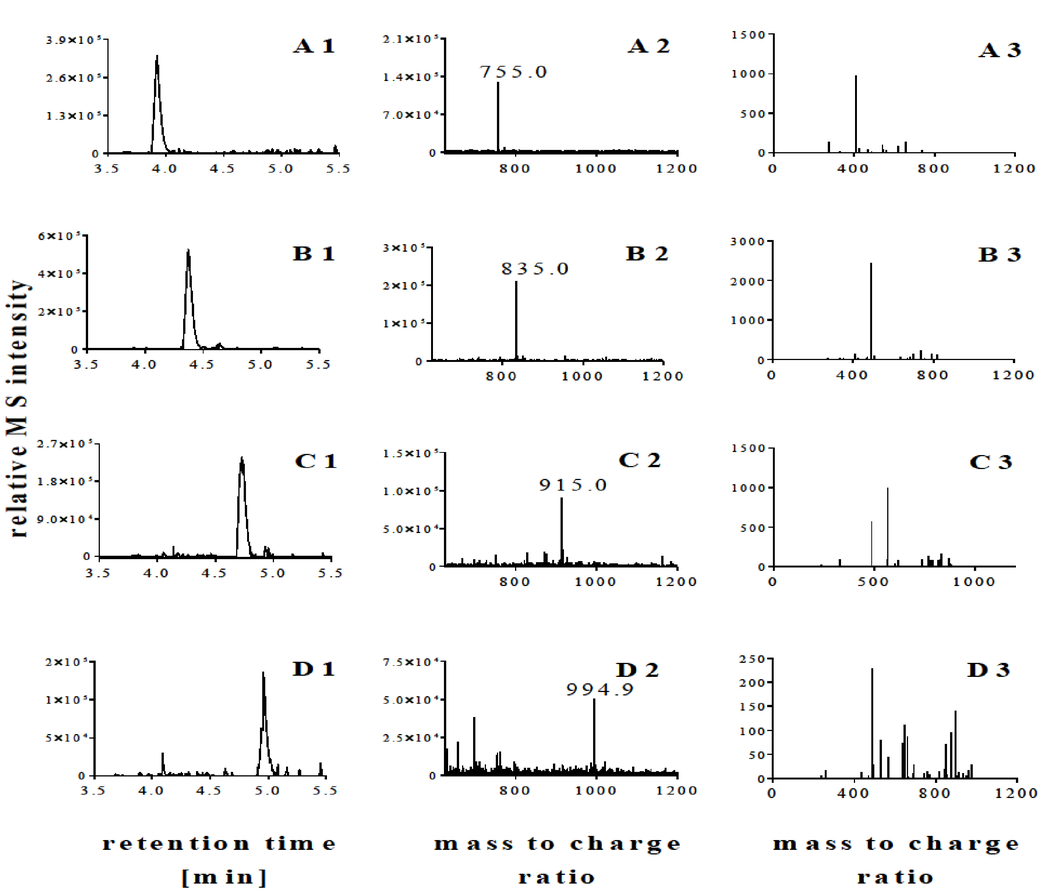

Figure. 2: Representative extracted ion chromatograms of endogenous diadenosine polyphosphates (**A1, B1, C1, D1**) as well as the corresponding negative ion ESI-MS (**A2, B2,C2, D2**) and ESI-MS/MS spectra (**A3, B3, C3, D3**).

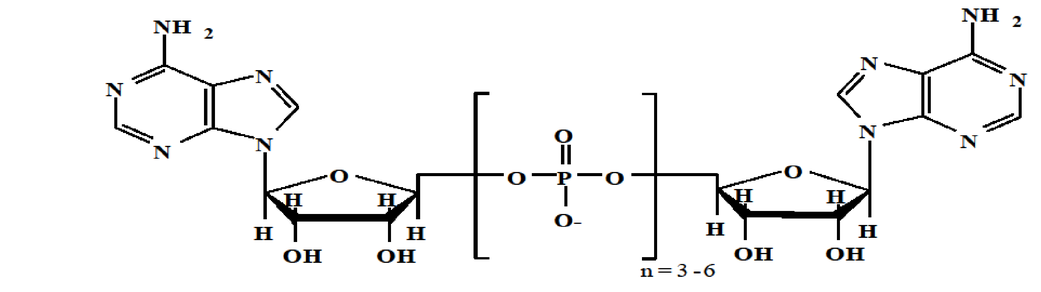

Figure 1: Molecular structure of diadenosine polyphosphates (Ap3–6A)

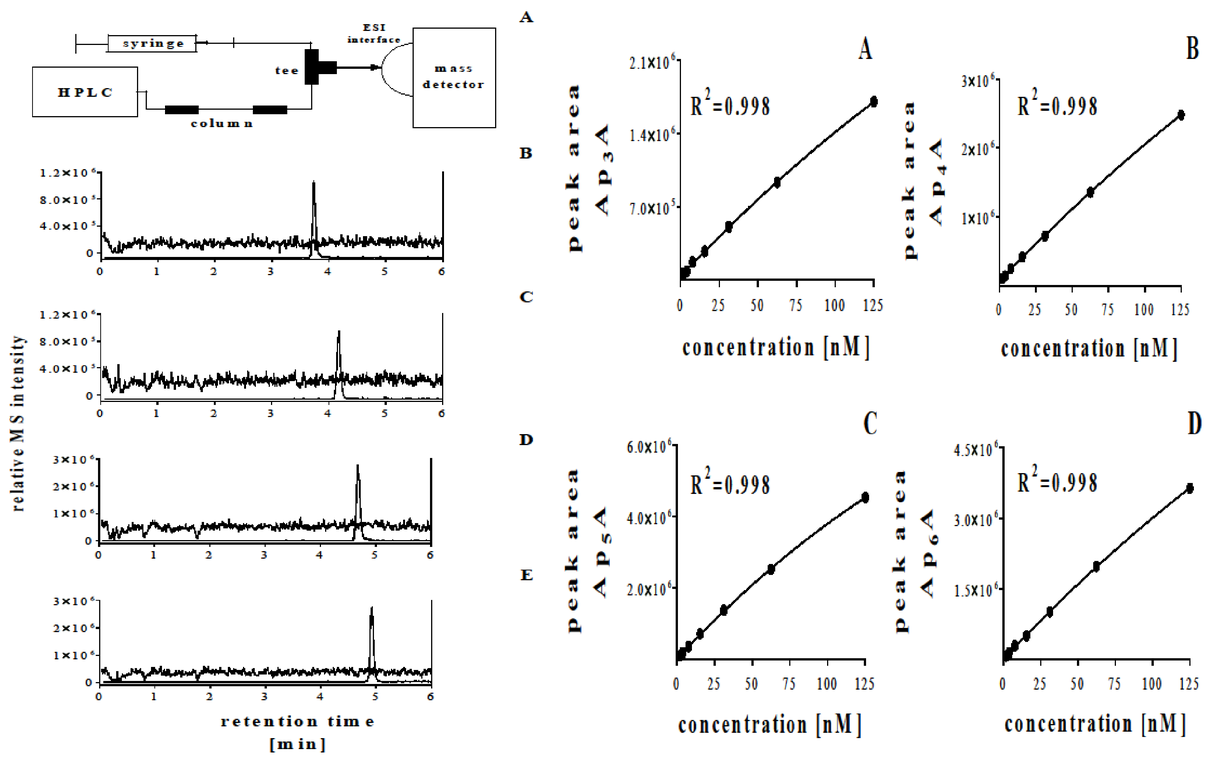

Figure 3: Investigation of ion suppression effects by the post column infusion method. **(A)** Scheme of the post-column infusion system; **(B)–(E)** overlay chromatograms showing the matrix effects due to endogenous plasma compounds for Ap3A **(B)**, Ap4A **(C)**, Ap5A **(D)** and Ap6A **(D)**.
Figure 4: Calibration curves of Ap3A **(A)**, Ap4A **(B)**, Ap5A **(C)** and Ap6A **(D)**

Table 1: Accuracy and precision of quality control samples

Analyte	QC	concentration (nM)	Intra-day (n=3) accuracy (%)[1]	Intra-day (n=3) precision (%)[2]	Intrer-day (n=9) accuracy (%)[1]	Intrer-day (n=9) precision (%)[2]
Ap_3A	LLOQ	1.9	109.6	5.1	106.6	9.6
	LQC	12.5	95.5	6.2	96.2	6.2
	MQC	50	100.3	3.8	102.0	4.0
	HQC	100	109.7	1.0	106.1	3.4
Ap_4A	LLOQ	1.9	108.2	1.7	102.4	10.5
	LQC	12.5	100.0	8.6	97.7	7.3
	MQC	50	101.9	2.5	102.9	1.6
	HQC	100	106.9	0.6	105.2	0.9
Ap_5A	LLOQ	1.9	100.6	11.4	105.4	11.4
	LQC	12.5	100.9	5.1	97.1	3.49
	MQC	50	97.7	3.9	97.8	3.2
	HQC	100	110.9	1.3	106.2	0.9
Ap_6A	LLOQ	1.9	106.3	6.4	108.9	8.7
	LQC	12.5	90.5	1.62	91.4	3.9
	MQC	50	107.0	2.42	101.0	3.2
	HQC	100	91.8	0.1	98.8	0.8

[1]calculated as (mean determined amount/nominal amount×100)

[2]calculated as % CV. (SD./mean)×100

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CONCLUSIONS

Since this method requires low volume of plasma to quantify the diadenosine polyphosphates, highly applicable for clinical applications and also for basic science to study its impact during pathophysiological conditions.

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