Rapid analysis of 11 energy-rich phosphate compounds using the SCIEX Triple Quad 6500+ system

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ABSTRACT

High-energy phosphate compounds refer to a class of phosphate compounds that can release a lot of energy during hydrolysis. Most of these compounds contain 1 to 3 phosphate groups, such as adenosine triphosphate (ATP). As the phosphate group in the compounds is easy to combine with trace metals in the system[1], these compounds always have poor peak shape and poor sensitivity when analyzed using liquid chromatography-mass spectrometry (LC-MS).
In this paper, the quantitative methods of 11 compounds containing high-energy phosphate bonds were established by a SCIEX Triple Quad 6500 + system, including CMP, dATP, dCMP, dCTP, dTMP, dTTP, UDP, UMP, AMP, GMP and IMP. The mobile phase system of the method is simple without adding ion pair reagent, which can reduce instrument contamination and increase the versatility of the method. In addition, the method has good sensitivity and reproducibility, and the linear relationship of each compound within the linear range is good.

INTRODUCTION

High-energy phosphoric acid compounds can release a lot of energy during hydrolysis. When these compounds are analyzed using LC-MS, the mobile phase additive options are limited, and the chromatographic peak shape is often suboptimal, resulting in poor analytical response and lower sensitivity than desired. Here, a quantitative method for analyzing 11 compounds containing high-energy phosphate bonds was established using a normal mobile phase instead of an ion-pairing agent. The limit of quantitation can reach below 0.1 ng/mL, and the detection limit of some compounds can be as low as 0.01 ng/mL.

MATERIALS AND METHODS

Sample preparation:
All the Individual standards need to be stored below -20℃ before use. The mix standards of 11 high-energy phosphoric acid compounds (CMP, dATP, dCMP, dCTP, dTMP, dTTP, UDP, UMP, AMP, GMP and IMP) were diluted with water. The commercialized standard stock solution contains sodium hydroxide to form sodium salts, which was directly diluted with pure water for standard curve injection analysis. The powder standard was dissolved in pure water. If the dissolution is not good, an appropriate amount of ammonium hydroxide can be added to assist in dissolution.

HPLC conditions:
A SCIEX ExionLC™ AD system with a Phenomenex Synergi 4 µm Fusion-RP, 150 × 3mm, 4 µM column at 40℃ with a gradient of eluent A (H_2O with 5 mM ammonium formate and 5 µM methylene diphosphate) and eluent B (95% acetonitrile with 5 mM ammonium formate and 5 µM methylene diphosphate) was used at a flow rate of 250 µL/min. The injection volume was set to 10µL and the LC gradient is 13 min (Table 1).

MS/MS conditions:
A Sciex Triple Quad™ 6500+ LC/MS/MS system with an IonDrive Turbo V ion source and electrospray ionization (ESI) probe was used. Eleven compounds containing high-energy phosphate bonds were detected using 2 MRM transitions per compound in negative polarity (Table 2).

Time(min)	Flow (mL/min)	Phase A (%)	Phase B (%)
0	0.25	99	1
2	0.25	98	2
5	0.25	90	10
8	0.25	10	90
9	0.25	10	90
9.1	0.25	99	1
13	0.25	50	1

Table 1. LC gradient conditions

Name	Q1	Q3	DP	CE
CMP	322.0	96.9/78.9	-40/-90	-25/-80
dATP	490.0	159.0/78.9	-40/-40	-30/-120
dCMP	306.1	194.9/78.9	-50/-50	-25/-60
dCTP	466.0	159.0/78.9	-40/-40	-35-120
dTMP	321.0	194.9/78.9	-50/-50	-25/-45
dTTP	481.0	159.0/78.9	-40/-40	-45/-120
UDP	403.0	159.0/78.9	-50/-50	-35/-100
UMP	323.1	96.9/78.9	-40/-40	-27/-80
AMP	346.1	134.0/96.9	-50/-50	-40/-36
GMP	361.9	210.9/150	-50/-50	-25/-35
IMP	347.0	134.9/96.9	-40/-40	-35/-31

Table 2. Compound parameters. MRM conditions for each of the analytes in the assay, two MRMs for each compound

RESULTS

A total of 11 compounds were analyzed by MRM acquisition. Concentration curves were generated across 10 different concentrations for each standard. All 11 compounds had good reproducibility within the range of the standard curve (n=7). The reproducibility for 6 consecutive ejections of 1 ng/mL standard was less than 5% for all 11 compounds. Most of the compounds had low limits of quantitation (LOQs), such as UDP (0.05 ng/mL) and UMP (0.05 ng/mL)（Table 3）.

We investigated the effects of different mobile phase additives and the use of different columns on the instrument sensitivity to the phosphate compounds. Methylene diphosphate appears to be a good additive to reduce metal adsorption on phosphate compounds, and it creates minimal background and minimal instrument contamination when added to the mobile phase at concentrations of less than 10 µM. Ammonium formate provides an appropriate pH for the compounds to bind on the column.

Name	Abbr	Concentration range (ng/mL)	R value	LLOQ (ng/mL)
Cytidine	CMP	0.2-100	0.999	0.20
Adenine triphosphate deoxynucleotide	dATP	0.1-100	0.999	0.10
2 '- deoxycytidine - 5' - monophosphate	dCMP	0.1-100	0.999	0.10
Cytosine triphosphate deoxynucleotide	dCTP	0.1-100	0.999	0.10
Thymine deoxynucleotide	dTMP	0.1-100	0.999	0.10
Thymine triphosphate deoxynucleotide	dTTP	0.1-100	0.999	0.10
Uridine diphosphate	UDP	0.05-100	0.999	0.05
Uracil glycoside	UMP	0.05-100	0.999	0.05
Adenine nucleotide	AMP	0.2-100	0.999	0.20
Guanine nucleotide	GMP	0.2-100	0.999	0.20
Hypoxanthine nucleotides	IMP	0.2-100	0.999	0.20

Table 3. LLOQ and linearity range of all 11 compounds.

Once the optimized mobile phases and columns were established, it was determined that this mobile phase system provides very good peak shape and sensitivity without impacting instrument uptime. The LC-MS/MS conditions described here provide a robust method for the study of phosphate-containing compounds. The extracted ion chromatograms of the 11 compounds at 1 ng/mL are shown in Figure 1, all the 11 compounds have good peak shape. All 11 compounds were continuously injected into 1 ng/mL concentration for 6 injections, and the RSD calculated using peak area was less than 5%, indicating good reproducibility of this method. The 11 calibration curves are shown in Figure 2, all the analytes have strong linearity with correlation coefficient (R) of 0.999 when using $1/x^2$ weighting.

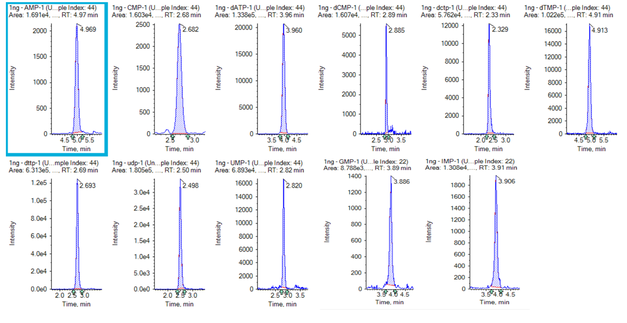

Figure 1. Extracted ion chromatograms (XICs) of the analytes at 1 ng/mL

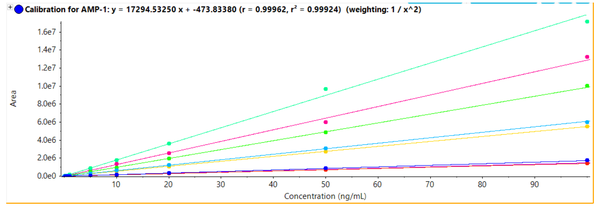

Figure 2. Strong linearity was achieved with a correlation coefficient (R) of 0.999 using $1/x^2$ weighting for each analytes.

CONCLUSIONS

A fast, robust, and reliable method, for the quantitation of energy-rich phosphate compounds was developed. The mobile phase system of this method is simple, and it does not need to add ion-pair reagents, which can reduce instrument pollution and increase the versatility of the method. In addition, the method has good sensitivity and reproducibility, and the linear relationship of all compounds is good within the linear range. Limits of quantitation (LOQ) of all compounds were found between 0.05 ng/mL and 0.2 ng/mL . All the analytes have strong linearity and good reproducibility and can meet the quantitative requirements of high-energy phosphate compounds in samples with different concentrations.

REFERENCES

[1]. Henryk S, Markus N, Marco H; Enhanced nucleotide analysis enables the quantification of deoxynucleotides in plants revealing connections between nucleoside and deoxynucleoside metabolism. THE PLANT CELL 2021: 33: 270–289.

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