Dual Strategy for 13C-Metabolic Flux Analysis of Central Carbon and Energy Metabolism in Mammalian Cells Based on LC-isoMRM-MS

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Introduction

Central carbon and energy metabolism are key metabolic pathways in biological science, thus it is important to be able to accurately measure their reaction rates. However, current methods of detecting metabolites via 13C-Metabolic flux analysis (13C-MFA) are limited by the instability of metabolites such as a-keto acids and phosphate compounds, leading to inaccurate measurements. This study hence presents a novel dual strategy method that aims to increase compound stability while maximising efficiency - N-Methylphenylethylamine (MPEA) is used to derivatize unstable metabolites while the remaining metabolites undergo simple extraction. This is the first instance of MPEA being used for the derivatization of phosphate groups.

Material & Methods

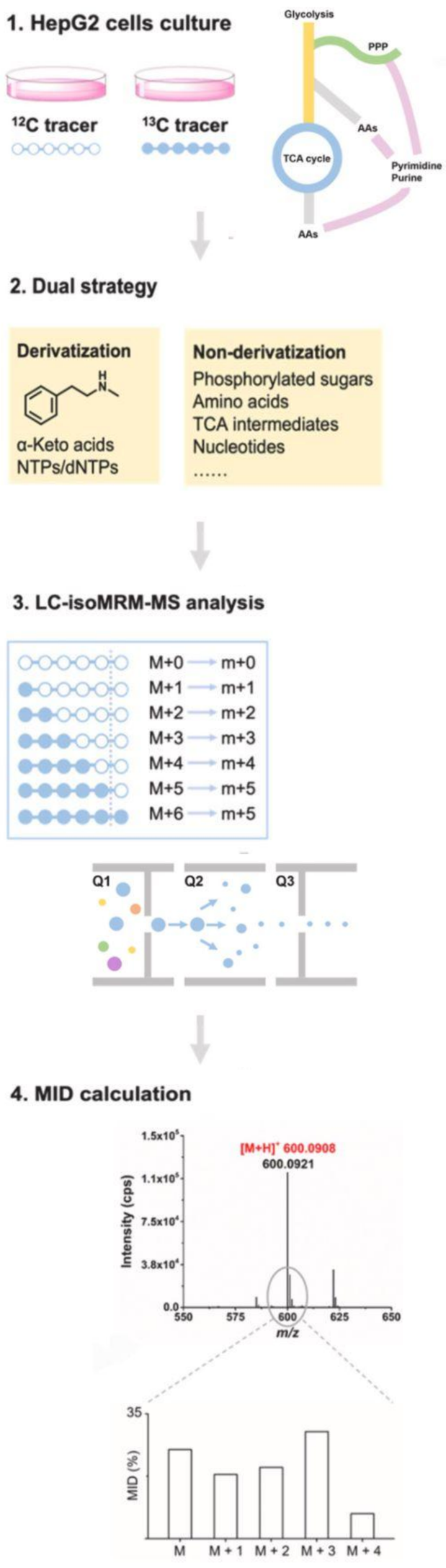


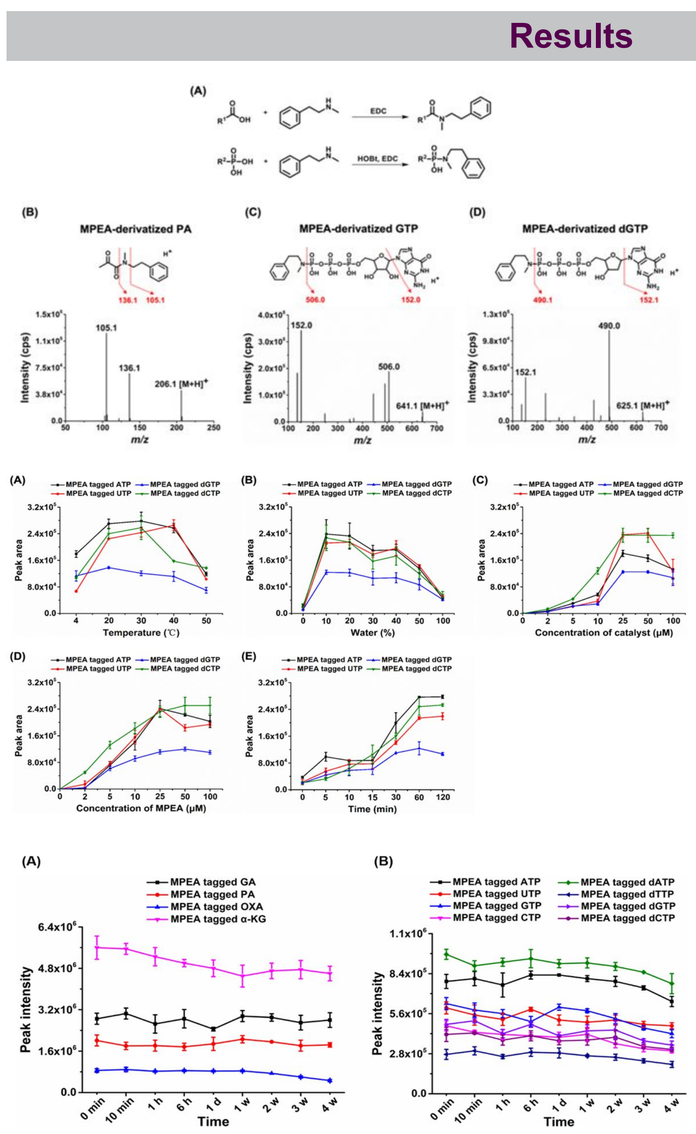
Fig 1. HepG2 cells was cultured with DMEM, followed by a 13C-labelling experiment to track how glucose is metabolized.

Fig 2. Dual strategy method used.
(1) Derivatization of a-Keto acids and phosphate metabolites (NTP/dNTPs) with MPEA under optimised reaction conditions.
(2) Non-derivatization of other metabolites using simple extraction.

Fig 3. Metabolites were directly analyzed using **Liquid Chromatography and Mass Spectrometry (LC-isMRM-MS) analysis.** Gradient elution method was used, where non-derivatized molecules are separated using an amide column, and derivatized molecules were separated using a C18 column. Separated molecules were then passed through an electrospray ioniser before entering the mass spectrometer which detected the mass-to-charge ratio.

Fig 4. Methodological Assessment & Data Analysis
Limit of detection was a signal-to-noise Ratio (S/N) of 3 and limit-of-quantification was S/N 10. Analyst 2.2.0 was used to extract information including accurate m/z, peak intensity, and retention time (RT) on the detected metabolites from raw data.

Results



Collision-induced dissociation was used to fragment derivatized molecules to aid in compound identification. 12 metabolites were detected from the MPEA-derivatization strategy.

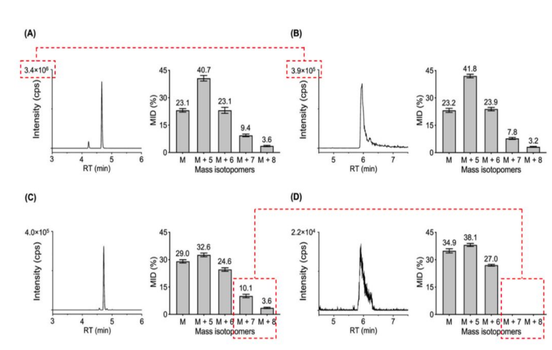
The derivatized phosphate molecules were derivatized with MPEA under different conditions to find the optimal reaction conditions. Under optimised conditions, the efficiency of MPEA and phosphate metabolites was greater than 90%.

MPEA-derivatized phosphate metabolites were stored at 4° C for 4 weeks and their stabilities were measured, showing remarkable stability, proving that this method is superior to traditional methods.

Detection of MPEA-derivatized α-keto acids improved by up to 2750- fold, and MPEA-derivatised NTPs and dNTPs also improved by almost two orders of magnitude. LODs of the non-derivatized metabolites were as low as 0.2 ppb. All 101 metabolites had intra- and inter-day relative standard deviations (RSDs) below 20%, suggesting that the method was reliable for the quantification of central carbon and energy metabolism.

MRM transitions of fumarate isotopomers.

	Name	Precursor ion	Product ion	RT (min)
Base peak	Fumarate	115 (M)	71 (m)	5.51
Isotopic peak_1	Fumarate_13C1	116 (M + 1)	71 (m)	5.51
Isotopic peak_2	Fumarate_13C1 (2)	116 (M + 1)	72 (m + 1)	5.51
Isotopic peak_3	Fumarate_13C2	117 (M + 2)	72 (m + 1)	5.51
Isotopic peak_4	Fumarate_13C2 (2)	117 (M + 2)	73 (m + 2)	5.51
Isotopic peak_5	Fumarate_13C3	118 (M + 3)	73 (m + 2)	5.51
Isotopic peak_6	Fumarate_13C3 (2)	118 (M + 3)	74 (m + 3)	5.51
Isotopic peak_7	Fumarate_13C4	119 (M + 4)	74 (m + 3)	5.51



First, the dual strategy was applied to detect metabolites in HepG2 cells, which allowed for the detection of 4 additional metabolites compared to the traditional method. Then, isotopic MRM transitions were generated, and dual strategy was applied to detect isotopologue metabolites in [U-13C]-glucose cultured HepG2 cells. Derivatization by MPEA significantly increased detection sensitivity of isotopic metabolites and yielded more consistent results than the traditional method. Hence, dual strategy is reliable and can be applied to intracellular 13C-MFA.

This dual strategy can be applied to investigate the metabolite differences between cancer and non cancer cells.

Conclusion

This study establishes the use of dual strategy for 13C-MFA using MPEA derivatization which significantly improved stability and detection of certain metabolites. This demonstrates the potential for dual strategy to facilitate accurate quantification of metabolites in biological systems.

References

Original paper: Zheng J, Yang J, Liang X, Fang M, Wang Y. Dual strategy for 13C-Metabolic flux analysis of central carbon and energy metabolism in Mammalian cells based on LC-isoMRM-MS. Talanta [Internet]. 2023 Aug 22;266:125074.
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