

Abstract No.1

Comprehensive MRM Analysis of Fat Soluble Vitamins including 25(OH)-Vitamin D3: Sensitivity and Selectivity by ESI LC/MS/MS

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Speaker invited by IONICS

INTRODUCTION: Analysis of vitamins is of importance in clinical diagnosis, food products and research, and the use of LC/MS/MS has supplanted the use of traditional technologies for vitamin analysis due to increased selectivity, sensitivity, and safety. In particular, vitamin D deficiency is common in North America and causes poor bone health, rickets/osteomalacia, and increased risk of various cancers. A method for the extraction and analysis by LC/MS/MS of the fat-soluble vitamins: A (retinol), D2 (ergocalciferol), D3 (cholecalciferol), E (α -tocopherol, γ -tocopherol), K1, and the structurally-related Coenzyme Q10 is reported here. The chromatographic method has been shown to separate endogenous interfering compounds from 25(OH)-vitamin D3 which is the major metabolite for determination of accurate Vitamin D status in the clinical setting.

METHOD :Mass Spectrometer: Analysis of vitamin samples was performed on an IONICS 3Q Molecular Analyzer using positive ion electrospray and monitoring MRM transitions for each vitamin standard and isotopically-labeled standards.

HPLC Conditions:A chromatographic method for separating fat-soluble vitamin standards from endogenous interfering compounds in human serum was established on a Shimadzu Prominence UFLC XR using a Kinetex PFP 100A 100X3 mm (2.6 μ m) column with a flow rate of 0.5 mL/min. The mobile phase was 4/96 water/methanol 0.1 mM ammonium acetate and 0.1% formic acid. The injection volume was 5 μ L for each sample.

RESULTS:The major fat-soluble vitamins were separated with a retention time range of 1 to 2.3 minutes, with coenzyme Q10 coming off the column last at 5 minutes.

Low pg/ μ L detections of the fat soluble vitamins demonstrate high versatility and sensitivity of IONICS 3Q Molecular Analyzer. LODs are at low fg/ μ L for vitamin K1, Coenzyme Q10, α -Tocopherol and γ -Tocopherol. Phylloquinone (K1) had excellent linearity ($R^2 = 0.9997$) over four orders of magnitude from 0.1 to 1000 pg/ μ L. For 25(OH)-Vitamin D3 analysis, 0.5 to 200 pg/uL was tested and found to be in a linear range ($R^2 = 0.9999$, CV <10%). Good peak shape and peak integration was obtained even at the near LOD of 1.92 pg/uL.

NOVEL ASPECT: A comprehensive LC/MS/MS method has been developed for the analysis of major fat-soluble vitamins including 25(OH)-Vitamin D3 without endogenous interferences.

Abstract No. 2

Evaluation of a New Prototype Accurate Mass System for Simultaneous Quantitative and Qualitative Bioanalysis and Metabolite Profiling

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Novel Aspect: Use of new prototype accurate mass analyzer with enhanced sensitivity, speed and quantitative linearity for PK studies under UHPLC conditions

Introduction: Combining quantitative analysis on the parent drug and qualitative analysis on the metabolites profile for early ADME studies has shown significant promise for improving the efficiency of drug discovery. Additionally, earlier information on the metabolite profile of lead compounds has gained increasing importance due to recent MIST guidelines. A number of different types of mass analyzers have been evaluated for these studies. Most of these studies have been focused on in vitro samples where the analytical requirements are generally less demanding than in vivo PK studies. In this study, we evaluate the sensitivity, linearity and speed of a prototype accurate mass system for Quant / Qual analysis of in vivo samples derived from rat blood/plasma under UHPLC conditions.

Methods: Methoxyfenoterol was administered to SD rats (IV 5 mg/Kg, PO 15 mg/Kg). Plasma samples were serially collected pre-dose and at 0.25, 0.5, 1, 2, 4, 6 and 8 hours post dose. For iv dosed, one additional time point at 0.0833 hour was also collected. Samples were protein precipitated and injected without pre-concentration. Samples were analyzed under UHPLC conditions on a Shimadzu Prominence XR system coupled to a prototype accurate mass system operated in ESI mode. Data acquisition was performed in the dedicated TOF MS mode and TOF MS with information dependant acquisition (IDA) and real time multiple mass defect triggering of product ion scans. Dedicated looped TOF MS and product ion scans were also performed to compare quantitative performance of the two scan modes.

Results: Linearity, sensitivity, and scanning speed performance were evaluated for the dedicated TOF MS mode as well as TOF MS with IDA. System was operated at a resolution of 30,000. Cycle times comparable to those used during traditional QqQ analysis were achieved, allowing for sufficient acquisition of spectra for quantification under UHPLC conditions. System performance demonstrated quantitative correlation in TOF MS mode of 3 orders of magnitude or more for methoxyfenoterol and fenoterol. The lower limits of quantification in extracted plasma were 0.5 and 1 ng/mL for methoxyfenoterol and fenoterol, respectively. The width of the extracted ion chromatogram (XIC) window was evaluated for optimal selectivity and sensitivity for parent compound in this in vivo matrix. In addition to quantifying parent, the in vivo data was interrogated for metabolites using mass defect filtering and sample / control comparisons. The major metabolite was the glucuronidation product and the demethylation product as also detected. The novel approach of applying multiple mass defect filters in real time to select parent ions for MS/MS during IDA significantly increased the efficiency of MS/MS data acquisition on relevant metabolites. Finally, estimation of metabolite quantity was performed by comparing the relative area counts in the accurate mass extracted ion chromatograms from the TOF MS data to that of the parent compound.

Abstract No. 3

Optimizing the ionization and chromatographic behavior of some water soluble vitamins for analysis by LC-MS

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-Novel aspect

A systematic approach was followed to evaluate the response of the vitamins in the ESI source with regard to solvent composition, acidity (pKa) and surface acitivity (octanol/water coefficient).

-Introduction

Besides instrumental parameters of the mass spectrometer, the physical and chemical nature of analytes has a big impact on the response of the analytes in the ESI source. Factors such as acidity, surface activity, solvation energy of the analytes can influence their response. In this paper we studied the ionization of the vitamins in different solvents and tried to correlate their response based on their surface activity.

- Method

Using infusion experiments, the ESI response of the various vitamins were evaluated in different solvents including acetonitrile (ACN), methanol, water/ACN mixtures containing formic acid and ammonium formate at different pH's. A PerkinElmer Flexar FX-15 LC pump with Flexar SQ 300 MS single quadrupole mass spectrometer were used for separation and detection of the vitamins. The vitamins were separated on a Brownlee ultra II aqueous column (1.9 μ m, 2.1X50mm) using various solvents conditions. The single quadrupole mass spectrometer was operated in positive mode in scan mode (100-400 m/z) and in selected ion monitoring mode.

- Detailed Results

Infusion experiments in methanol and acetonitrile showed the vitamins to be more responsive in methanol because of the protic nature of methanol. However, there was no big difference in analyte response at 50% or greater concentration of water/methanol mixtures and in water/acetonitrile. The highly protic nature of water equalized the differences between the methanol and acetonitrile. Response of Riboflavin and pantothenic acid was significantly improved when the compounds were analyzed at a pH below the pKa of the compounds suggesting the gas phase chemistry of these compounds in the ESI source was similar to their solution phase chemistry. However, niacinamide, pyridoxine and thiamine did not show a significant increase in response upon lowering the pH of the solutions below their respective pKa's thereby suggesting, the gas phase chemistry of the molecules is different than their solution phase chemistry. The response of pyridoxine, niacinamide, pantothenic acid and riboflavin correlated with octanol/water coefficients suggesting the compounds with greater non-polar nature have higher affinity for the electrospray droplet surface and hence higher ionization efficiency. The optimum ESI conditions for ionizing the vitamins were not optimal for separating the compounds on column. Hence, a compromise for optimal LC separation versus optimal ESI response had to be worked out.

-Conclusions

Due to the complexity of the ESI response, it is difficult to predict the response of compounds in the ESI source. The analyte acidity (pKa) can be a first step to predict the response in positive

versus negative mode. However, with some vitamins such as niacinamide, protonation was observed at pH higher than its pKa suggesting there are other mechanisms that may explain ESI response. Many of the vitamins studied showed positive ESI response correlation to the octanol/water coefficient suggesting, the analyte hydrophobicity can be useful tool for predicting ESI-MS response.

Abstract No. 4

Determination of Levels of Volatile Methyl Silicones in Air Samples from across Canada

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Novel Aspect

Determination of novel and emerging contaminants in urban, rural and pristine settings, where concentrations ranged six orders of magnitude

Introduction

Cyclic volatile methyl silicones (cVMS) are high volume chemicals that are used in a wide variety of industrial applications and personal care products. Their physical chemical properties such as high volatility along with widespread application indicate that these compounds can be found everywhere in the environment. This has raised concerns in North America and Europe. As a result, potential risk from these compounds was assessed by EU and Canada. Since cVMS preferentially partition into the atmosphere, initial method development focused on a GC/MS based method suitable for the determination of cVMS in air from various locations across Canada.

Methods

Air sampling was accomplished by pulling air through Tenax sorbent at 50-100 mL/min using SKC PCXR4 pumps. Sorbent tubes were analyzed using a Gerstel TDS3 system interfaced to an Agilent 6890N/5975B GC/MSD via a CIS4. The TDS3 was operated in splitless mode held at 20°C for 0.5 minutes, then raised to 260°C at 30°C/min and held for 3 min. During TDS heating, CIS4 was held at -10°C. After TDS heating, the CIS was raised to 275°C at 10°C/min and held for 3 minutes GC oven temperature was held at 35°C for 1 min, raised to 195°C, at 20°C/min, then heated to 285°C at 10°C/min, and held for 3 min. The MSD was operated in SIM/Scan mode

Results

Since cVMSs are used in personal care products, and indoor building air is re-circulated continuously, elevated background concentrations of CVMS has been observed in laboratory and indoor air. As a result, background contamination is the main challenge with the analysis cVMS. In this study, sample contamination was minimized by using a fully automated thermal desorption GC/MS technique. Automation makes it possible to minimize sample manipulation during field sampling and laboratory analysis. Sorbent tubes filled with Tenax and compact battery operated pumps were shipped in small suitcases to remote areas such as the Arctic where air samples could be collected very efficiently. Air samples were collected from urban areas as well as remote Arctic regions. The total concentration of VMS in biogas from four WWTP in Southern Ontario was $2.5 \times 10^4 \mu\text{g}/\text{m}^3$. Decamethylcyclopentasiloxane (D5) was measured in highest concentrations followed by octamethylcyclotetrasiloxane (D4) and

dodecamethylcyclohexasiloxane (D6). These levels are significantly higher than those observed around the aeration tank in WWTPs where the cVMS concentrations were around $5\mu\text{g}/\text{m}^3$. Levels of cVMS in urban areas in Southern Ontario ranged between 0.04 and $0.18\mu\text{g}/\text{m}^3$, in remote areas such as Lac St. Pierre in Quebec ranged between 0.01 and $0.035\mu\text{g}/\text{m}^3$ and Turkey Lakes in Northern Ontario ranged between 0.01 and $0.042\mu\text{g}/\text{m}^3$ and for Resolute Bay in Nunavut ranged between not detected (n.d.) and $0.013\mu\text{g}/\text{m}^3$ and for Little Fox Lake in Yukon ranged between 0.005 and $0.012\mu\text{g}/\text{m}^3$. In samples from Resolute Bay, D4 was the predominant congener indicating long range transport of VMS. To the best of our knowledge these are the first determinations of the levels of VMS in the Arctic.