INTRODUCTION

A standard mixture containing 24 plant metabolites, predominantly flavonoids and flavonol glycosides was prepared and analyzed using UPLC-MS/MS and monolithic LC-MS/MS methods. Two modes of data acquisition have also been compared: data dependent analysis (DDA) and MSE.

To demonstrate the suitability of these methods for analysis of real plant extracts both methods were used to analyze a butanol extract of the aerial parts of Genista tenera.

Flavonoids and flavonol glycosides constitute a large, diverse class of secondary metabolism diversity is attributed to various functional groups (e.g. -OCH3, -OH) substituted on the flavonoid ring. A standard mixture containing 24 plant metabolites, predominantly flavonoids and flavonol glycosides were analyzed using UPLC-MS/MS and monolith LC-MS analysis.

Affiliations

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METHOD

Samples (5 μL) were injected onto the columns and eluted using an optimized gradient of water containing 0.1% formic acid, and acetonitrile containing 0.1% formic acid. Data were acquired using an external reference to ensure good mass accuracy. Data acquisition was achieved using either MS/MS (Figure 2) or using traditional DDA.

LC and MS conditions

LC System: Waters® ACQUITY UPLC® System
MS/MS System: Waters® Micromass TQ System
Column: ACQUITY HSS T3 Column (2.1 x 100 mm, 1.8 μm)
Column Temp: 35°C
Flow Rate: 0.6 mL/min
Mobile Phase A: H2O (0.1% FA)
Mobile Phase B: Acetonitrile (0.1% FA)
Gradient: 10-15% B/8 min, 25-70% B/2 min, 90% B/1 min.
Monolithic Column: Hans Chromatography Performance
Column Temp: 35°C
Flow Rate: 2 mL/min (0.6 mL/min to MS)
Mobile Phase A: H2O (0.1% FA)
Mobile Phase B: Acetonitrile (0.1% FA)
Gradient: 10% B/1 min, 10-20% B/7 min, 20-90% B/4 min, 90% B/1 min.
MS System: Waters Q-Tof Premier Mass Spectrometer
Ionization Mode: ESI Negative
Desolvation Temp: 350°C
Desolvation Gas: 900 L/Hr
Source Temp: 120°C
Collision Energy: 100-160 eV

RESULTS AND DISCUSSION

A traditional C18 LC method for the analysis of this plant extract took 20 minutes (A Rauter et al. J. Chromatogr. A 2005, 1089, 59-64). Using both our chromatographic methods the analysis takes 10 minutes.

Many more peaks were identified using UPLC-MS compared to monolithic LC/MS.

Approx. 150 MS/MS experiments were performed during the UPLC/MS/MS analysis and even more components were detected using Waters MetaboLynx to process the MS/MS data we are still analyzing this data—

Table 1. Screening the 24 components of our mixture, their elemental formulas, m/z value for [M-H]-, the retention times, MS/MS fragmentation data acquired using DDA and by MetaboLynx processing of the MS2 data.

- Using the UPLC-MS method more components were identified by using the monofunctional MS (Fig. 3).
- The MS2 method found more peaks times comparable to those obtained using UPLC-MS a flow rate of 2 mL/min was used. Metabolite flow was split to be ESI compatible.
- Similar fragmentation is obtained using MS2 and conventional MS/MS (Fig. 4).
- 23/24 components of the mixture were detected using MSE/MSD (DDA).
- 22/24 components of the mixture were detected using monolithic LC/MS.
- 20/24 components of the mixture were detected using monolithic LC/MSE.

MSE (Figure 2) or using traditional DDA.

Conclusions

- The chromatographic methodologies used were compatible with both DDA and MSE.
- MetaboLynx UPLC/MS2 enabled identification of the largest number of components in the standard mixture and in the plant extract.
- Metabolomics software is heavily used in drug metabolite identification. This study shows that it is also able to perform metabolite identification. A particularly useful feature in the ability to deconvolute the neutral loss information from the high and low energy acquisitions, which enables flavonoid glycosides to be identified by their characteristic neutral losses of the glycosidic residue.

APPLICATION TO REAL PLANT EXTRACTS: GENISTA TENERA

- To achieve monolithic LC analysis times compatible with the monolithic method (Fig. 3).
- The monolithic method (Fig. 3).
- MetaboLynx enabled the user to easily data mine the information generated by the MS2 approach.