

AN APCI – LC-MS/MS METHOD FOR RAPID QUANTITATIVE ANALYSIS OF TEN PHYTOESTROGENS IN HUMAN URINE

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The importance of dietary intake of phytoestrogen-rich soy protein-containing foods as a factor in women's health has been supported by a wealth of biological activity data gained in the last 10 years. One of the main difficulties with this area of research is the quantitative validation of a patient's dietary exposure to phytoestrogens for correlation with the information obtained by questionnaires. The aim of this project was to develop a routine LC method for the simultaneous analysis of a number of phytoestrogens (isoflavones & lignans) in the urine of women with breast cancer. Available methods^{1,2} using LC with UV detection have the sensitivity for analysis of formulations, but require MS to detect these compounds in biological fluids. We have developed the use of APCI MS/MS detection to dramatically reduce the analysis time while retaining specificity to quantify a large number of compounds simultaneously. The compounds of interest were excreted as glucuronides and sulfates, so that hydrolysis with β -glucuronidase was performed prior to isolation using C₁₈ solid-phase extraction cartridges. The sample was reconstituted, injected onto a reversed-phase column and eluted with a methanol/water gradient. The MS was operated in positive ion, SRM mode isolating precursor & product ions, as shown in the example below. Column switching was employed to minimize ion source contamination during the analytical studies. The cycle time for each sample is ~12min. The method developed resulted in interday and intraday CV's of < 10% for all compounds studied, with recoveries of > 90% for most analytes from urine. The phytoestrogens (isoflavones & lignans) determined by this method were, dihydrodaidzein, enterodiol, dihydrogenistein, daidzein, enterolactone, equol, genistein, O-desmethyl-angolensin, formononetin & biochanin A.
