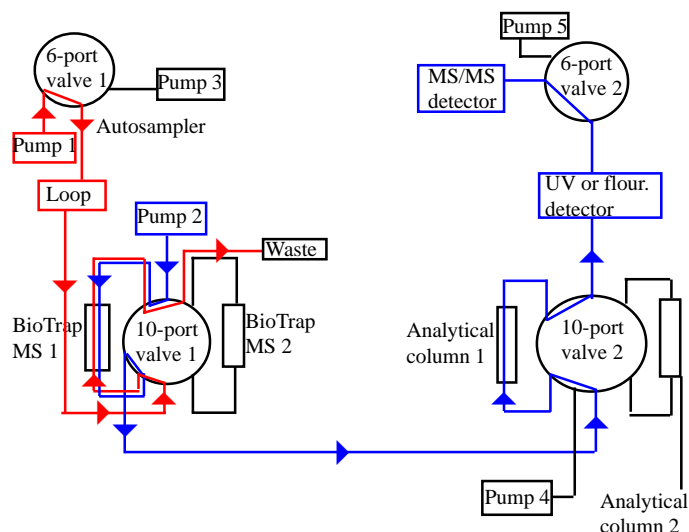


Automated on-line extraction from cell cultures using BioTrap 500 MS

Two highly automated, rapid and sensitive HPLC assays using on-line sample enrichment on BioTrap MS extraction columns have been published by G. Friedrich et al. The two methods have been published in *J. Chromatogr. B*. The first article entitled "Determination of lonazolac and its hydroxy and O-sulfated metabolites by on-line sample preparation liquid chromatography with fluorescence detection" was published in vol. 766 (2002) 295-305. The second article entitled "Determination of testosterone metabolites in human hepatocytes. 1. Development of an on-line sample preparation liquid chromatography technique and mass spectroscopic detection of 6 β -hydroxytestosterone" was published in vol. 784 (2003) 49-61.

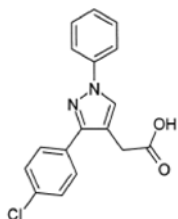
This on-line technique takes advantage of sample extraction and concentration on a biocompatible extraction column (**BioTrap MS**) allowing interfering larger compounds to be washed out while retaining the compounds of interest. The trapped compounds of interest are then backflushed onto an analytical column for separation and detection. The extensively automated procedure allows a high throughput of samples. At first sight the method might seem complicated, however once established it is a powerful alternative to time consuming conventional extraction procedures. The method described in these two publications are using an exceptionally automated method, however the usual and simple approach to using BioTrap columns can be found on www.chromtech.co.uk/biotrap. In the simple approach an ordinary HPLC system is used together with an extra 6-port valve and an extra pump. Friedrich et al have used two extra 6-port valves and two 10-port valves, as well as four extra pumps. They also use two BioTrap 500 MS columns (20x4 mm) and two analytical C18 columns.



The sample is injected, using Pump 1, via the autosampler (6-port valve 1) through 10-port valve 1 onto BioTrap MS 1, where the compounds of interest are trapped and unwanted proteins etc. are eluted to waste (red line). After the extraction sequence, both 6-port valve 1 and 10-port valve 2 are switched. In this way the compounds of interest trapped on BioTrap MS 1 will be backflushed onto analytical column 1 (using Pump 2) for subsequent separation and detection (blue line). The loop and BioTrap MS 2 are washed using Pump 3. Analytical column 2 is washed and equilibrated using Pump 4. The ion-source in the MS-detector is washed using Pump 5. Then the process is repeated with a new sample injected and trapped onto BioTrap MS 2 and backflushed to analytical column 2 for separation and detection, while BioTrap MS 1 and analytical column 1 are washed.

This method permits rapid processing and quantification of very large sample numbers and is a powerful alternative to more conventional time-consuming procedures. The validation data shows that the method provides reliable results. The experimental design can be used for quantitative determination of a wide range of biologically active compounds including pharmaceuticals in plasma, urine and tissue as well as milk, fermentation broth and other complicated matrices.

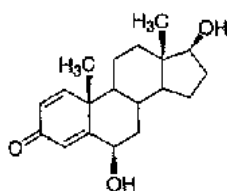
Lonazolac



Extraction mobile phase: 10 mM TEAF pH 5.5
 Flow rate: 3.2 ml/min
 Extraction time: 1 min.
 Sample volume: 50 μ l cell culture

Analytical mobile phase: gradient
 A: 10 mM ammon. acetate pH 8.5
 B: Methanol:2-propanol 9:1
 Flow rate: 0.2 ml/min
 Detection: Fluorescence ex. 273 nm em. 385 nm + MS/MS with ESI in SRM mode. Negative ion mode.

6 β -hydroxytestosterone



Extraction mobile phase: 10 mM TEAF pH 6.0
 Flow rate: 3.2 ml/min
 Extraction time: 2.5 min.
 Sample volume: 100 μ l artificial cell culture

Analytical mobile phase: gradient
 A: H₂O/MeOH/THF 73/20/7
 B: Methanol/THF 93/7
 Flow rate: 0.2 ml/min
 Detection: UV254 nm + MS/MS with APCI in SRM mode. Positive ion mode.

PLEASE NOTE: This is an exceptionally automated method. Usually BioTrap MS is used in an ordinary HPLC system equipped with an extra 6-port valve and a simple additional pump for the extraction mobile phase.

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