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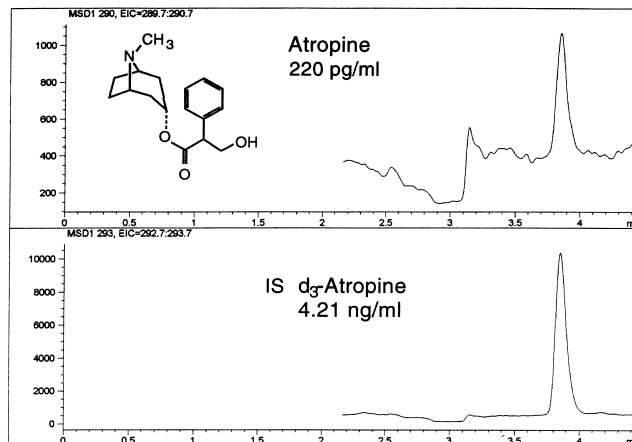
BioTrap 500 MS - a new way to fast bioanalysis

The popularity of the **BioTrap** columns increase continuously worldwide, as many bioanalytical chemists have discovered the time saving possibilities, i.e. the advantage of integrating the extraction and the analytical step. Today bioanalytical chemists are skilled in the use of column-switching systems and are able to utilize this technique.

Bioanalysis of drugs and related compounds in plasma/serum is one of the major areas in the analytical activities on pharmaceutical companies and hospital labs. A lot of money and time could be saved if these activities are performed in a rational way. The development of the **BioTrap MS** column has opened the way to strongly rationalize the bioanalytical activities when these columns are combined with a selective detector like for example the MS, MS/MS, fluorescence, EC etc. Using the **BioTrap MS** column together with a short analytical column and MS detection give the possibility to perform bioanalysis in a totally automated way, where the extraction and the analytical separation is integrated. In an optimized procedure the analysis of such a sample will only take 2-4 min., including both extraction and analysis. No manual handling is necessary in this procedure, the labdata system can control the whole procedure. Below is an example of the analysis of atropine in human serum using **BioTrap MS** and MS-detection. Please note that the total time of extraction and analysis is only 5 min. although a 150 mm long analytical column is used. A shorter analytical column would reduce the total time of analysis to only about two minutes.

Applications and more information on the **BioTrap** columns can be found on:

www.chromtech.se/biotrap



Extraction column: BioTrap 500 MS 20 x 4.0 mm
Mobile phase: 10 mM ammonium acetate, pH 10.0
Analytical column: C18 150 x 4.6 mm + guard
Mobile phase: 25 % acetonitrile in 50 mM formic acid

It is very simple to develop methods on this system since general extraction methods are used for both acidic and basic drugs.

Method development with the BioTrap 500 MS

1. Choose a detection method, MS or MS-MS
2. Develop a preliminary analytical method. Choose a column and a mobile phase composition giving a good chromatographic performance
3. Connect the extraction column and the analytical column to the switching valve. Extract the analyte using one of the general extraction mobile phases

Basic compounds: 4% 2-propanol in 10 mM ammonium ac. pH 10

Acidic compounds: 4% 2-propanol in 100 mM formic acid pH 2.4

4. Optimize the system.(changing anal. column, adjust the mobile phase composition).

5. Validate the method

For basic compounds, **note** that the analytical column must tolerate the extraction mobile phase pH of 10, as a plug of the extraction mobile phase will be transferred onto the analytical column with each sample.

A rapid automated HPLC method for the determination of benzodiazepines in plasma and serum using BioTrap 500 MS

C. Staub et al at Institut Universitaire de Médecine Légale in Geneva in Switzerland have published an article in Journal of Chromatography B, vol. 742, pp. 381-390 (2000) entitled “*High-performance liquid chromatographic method for the determination of benzodiazepines in plasma or serum using the column-switching technique*”. The article describes a method for the **simultaneous** determination of five frequently prescribed benzodiazepines:

- clonazepam
- diazepam
- flunitrazepam
- midazolam
- oxazepam

In the Experimental part of the article is a detailed description of the instrument setup. 50 µl plasma samples are injected into the **BioTrap 500 MS** column, 20x4.0 mm.

The **extraction mobile phase** consists of a 30 mM potassium phosphate buffer pH 7.2.

The **analytical mobile phase** is a linear gradient of 30-35% acetonitrile in 20 mM potassium phosphate buffer pH 2.1.

The method is optimized regarding acetonitrile content, buffer pH, buffer concentration, temperature and flowrate.

Validation of the method is performed on all the compounds at different concentrations.

The **linearity** was measured at 50 to 1000 ng/ml with correlation coefficients of 0.997-0.998.

Method **precision** was determined by measuring repeatability (intra-day precision) and reproducibility (between-day precision) of peak areas. RSD-values were around 1% for concentrations around 500 ng/ml and around 15% for concentrations around 50 ng/ml.

The **recoveries** were around 98% for all the benzodiazepines studied.

The **LOD** (limit of detection) was 15 ng/ml for clonazepam, flunitrazepam and midazolam, 18 ng/ml for oxazepam and 24 ng/ml for diazepam. **LOQ** (limit of quantification) was 50 ng/ml for clonazepam, flunitrazepam and midazolam, 80 ng/ml for diazepam and 60 ng/ml for oxazepam.

The **accuracy** of the whole procedure was verified with a certified standard serum. Excellent agreement with the certified values was obtained. The chromatogram at the bottom of the page is a certified serum at levels between 50 and 600 ng/ml using the BioTrap 500 MS extraction column connected with an analytical column via a 6-port valve.

If you need a copy of the article, please email support@chromtech.co.uk.

The **BioTrap 500 MS** columns are available in the following dimensions:

- 20x4.0 mm (BMS204K, BMS204C)
- 13x4.0 mm (BMS134K, BMS134C)
- 20x2.0mm (BMS202K, BMS202C)

