New Fully Automated System Simplifies Dioxin Sample Preparation, Saves Time and Reduces Costs

Current automated systems for dioxin sample preparation have the disadvantage that the consumables are very expensive and the solvent consumption is often higher than for manual processing. For this reason, even large international laboratories often revert back to using manual methods. These are however extremely complex, require a lot of time to carry out, and tie up staff. In order to reduce processing times at his Institute, Dr. Thorsten Bernsmann of the Chemical and Veterinary Analytical Institute Münsterland-Emscher-Lippe (CVUA-MEL), decided to convert the manual preparation method used at that time into an automated process. This project was to be realised in co-operation with the LCTech GmbH, an expert company in the field of automated sample preparation. The DEXTech™ system can be used universally for all matrices, is equipped with ready-to-use columns and also reduces the processing time from raw extract to fractions to only 96 mins. Including conditioning, the system uses less than 640 mL solvents. In addition, the DEXTech™ device is not tied to the use of the CVUA-MEL developed standard method alone, and can be employed even more flexibly.

> *"Method development is no longer necessary. However, the system allows to adapt individual processing steps or to program a new method via touch screen, such that the device can be used very flexibly"*

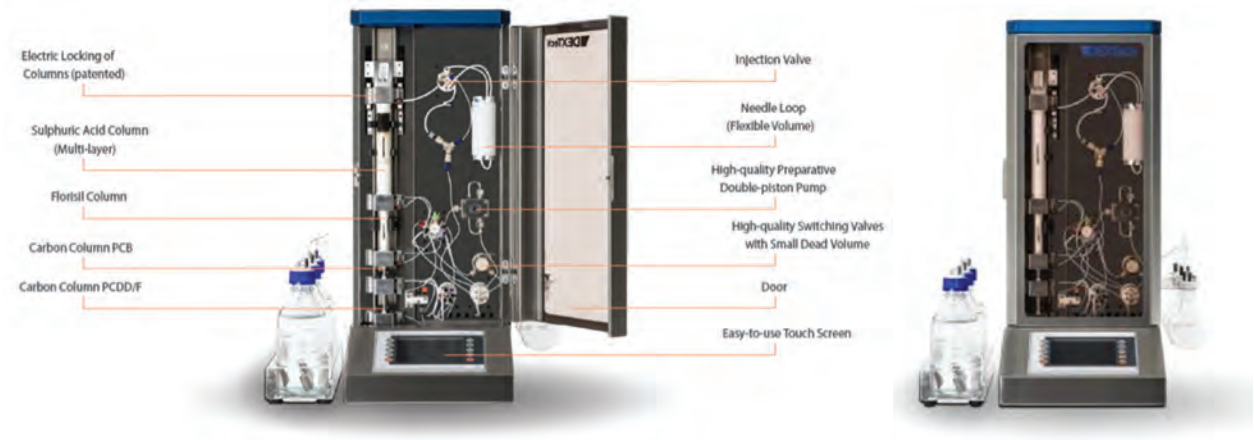

Figure 1
Dr. Thorsten Bernsmann of the CVUA-MEL developed together with LCTech GmbH an automated system for dioxin processing. The DEXTech™system can be used universally for all matrices, without prior clean-up. It is equipped with ready-to-use columns and reduces the processing time from raw extract to fractions to only 96 mins.

"There are different approaches available for dioxin processing. Basically, the process may include a GPC unit, a silica gel column, a silica gel column impregnated with sulphuric acid, a Florisil column, an alumina column as well as carbon columns of different activity", explains Dr. Thorsten Bernsmann, Head of the Dioxin and HPLC-MS/MS Department of the CVUA-MEL. When processing is conducted manually, extracts need to be evaporated before a solvent exchange can be carried out. "This can lead to a loss of analytes or to the introduction of contaminants," says Bernsmann. "In addition, dioxin processing involves probably the most complicated sample preparation I know. Up to 20 g of sample need to be concentrated to 20 µl. This corresponds to a concentration factor of 1000; hence the process takes up a considerable amount of time."

Project in co-operation with LCTech: Transfer to an automated system

The automated systems that had been on the market for a few years were not deemed to be viable alternatives by the CVUA-MEL scientists: Over and again, the Bernsmann team encountered during their evaluations the problem of very expensive consumables and a high solvent consumption, which by far exceeded that needed for their own manual processing. "In addition, enquiries amongst other users highlighted that the systems were maintenance-prone, took up a lot of space and needed to be placed underneath an extraction hood. All these points prevented us from the use of such a system to date." Instead, their own methodology of manual processing has been developed over the years with the development potential now fully exhausted to be able to process the samples as fast as possible.

In order to find a way to shorten the processing times even further, Bernsmann established contact with LCTech GmbH in the Bavarian Dorfen. After some discussions, it was decided that together with the LCTech experts in the field of sample preparation systems, the manual CVUA-MEL method would be transferred to an automatic system, which would forgo the problems of other existing systems. "During the development we needed to merge the best analytical approach with what was technically possible," explains Bernsmann. "One of the biggest challenges was to successfully implement the solvent exchange option between the individual column combinations in an automated system."

Author/Contact Details:
Dr. Thorsten Bernsmann
(The Chemical and Veterinary Analytical Institute Münsterland – Emscher – Lippe (CVUA-MEL);
Maximilian Baumann
LCTech GmbH
Bahnweg 41
84405 Dorfen / Germany
Tel: +49 8081 9368-0
Email: info@LCTech.de
Web: www.LCTech.de

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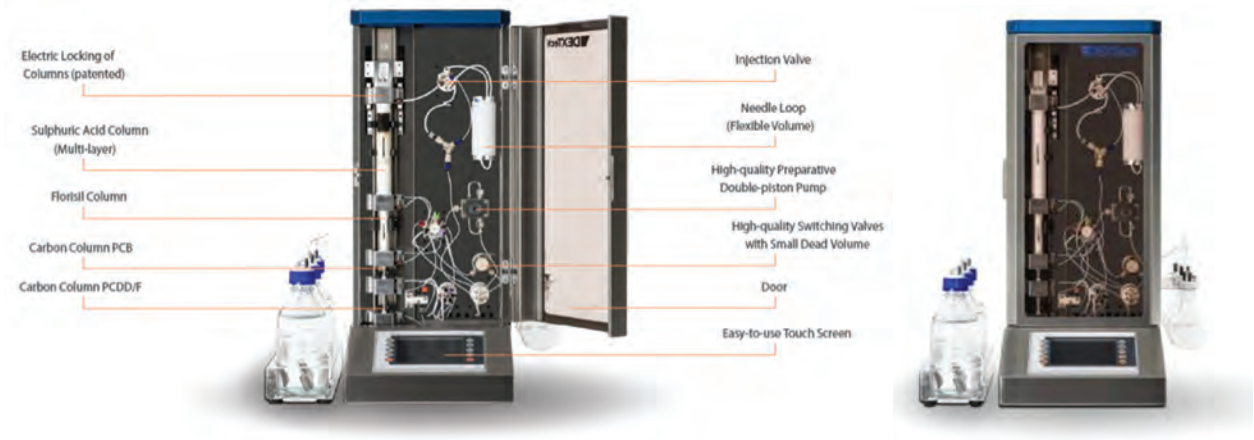

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Figure 2
The device, which emerged from the collaboration, is characterised by a particularly simple mode of operation. Preparatory work includes only the connection of collection vessels to the output lines and clipping the ready-to-use columns into the holder – the other system's standard screw connections are omitted, the locking is done by the system.

Figure 3
Injection into the sample loop is the only manual step of the entire preparation process, everything else runs fully automatically after you start the device.

Low detection limit through high fat content

Figure 4
The DEXTech™ system consists of, (from left to right) a silica gel column impregnated with sulphuric acid, a Florisil column, and two carbon columns of different activity.

Figure 5
In the silica gel column, fats and other substances will be oxidised. Depending on the fat matrix, the DEXTech™ can process up to 5 g fat fully automatically. Since the column body is made from glass, the user can even optically control the processes. (From right to left: 1 g; 1.5 g; 3 g fat).

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Afterwards, the inserted columns are conditioned and the fat/solvent mixture is put onto the first column, a silica gel column, which is sulphuric acid impregnated. Here, fats and other substances will be oxidised. Depending on the fat matrix, the DEXTech™ device can process up to 5 g fat fully automatically. Generally, 2 g fat is sufficient to achieve the required low detection limits. "Dioxins and PCBs stay intact on the sulphuric acid column and are rinsed with hexane onto the second column, the Florisil column," explains Bernsmann. On this column PCBs and dioxins are separated. The latter remain with hexane on the Florisil column, whilst PCB is transferred onto the first PCB-carbon column. There, the non-ortho-PCBs are retained with hexane, while the mono-and di-ortho-PCBs are collected in the first fraction. The non-ortho-PCBs are eluted from the carbon column with toluene using the reverse flow or backflush procedure.

Optimal Separation and Fractionation

"What's new is the transfer of the dioxins from the Florisil-phase with dichloromethane/hexane (1:1) to another carbon column using the reverse flow method. Through this method much solvent is saved and the dioxin fraction can be cleaned once more", explains the expert. The dioxins stick to the second carbon column, interfering substances can be removed with the solvent. From this carbon column, the dioxins will then be eluted again via backflush with toluene. The resulting three fractions only need to be evaporated and then measured. Owing to the optimum separation and fractionation of the dioxins and the different groups of PCB, a superimposition of the toxicologically particularly relevant non-ortho-PCB with mono-ortho- and di-ortho-PCB is avoided during the subsequent chromatographic analysis, and an incorrect evaluation is prevented from the start. From raw extract to the finished fractions, this process takes only 96 mins, including drying with nitrogen. The reaching of the program end can be made audible with the sound of a signal tone.

This universal method is suitable for food and feed samples as well as for environmental samples and enables any matrix to be processed. The method is pre-set in DEXTech™ and therefore can be easily applied to all. "Method development is no longer necessary. However, the system allows to adapt individual processing steps or to program a new method via touch screen, such that the device can be used very flexibly," says Bernsmann. A conversion from a 4- into a 3-column system is possible; dummy columns are included specifically for this purpose.

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Low solvent consumption and elimination of labour-intensive steps

Applying the standard method, the DEXTech™ uses less than 640 mL solvent including column conditioning, which is a huge saving compared to manual processing, and also to other systems. The low consumption is supported by specially constructed, rotatable switching valves with dead space free passages, which also prevent clogging of the system. A carry-over from one sample to another is not possible, as for example the sample loop can be rinsed very fast and effectively in the process. Compared to the manual methods, evaporation steps between sample loading and elution are negated with the DEXTech™. Preliminary tests of the materials used as well as filling, preparing and rinsing of the columns are no longer necessary. Consequently, all labour and time intensive steps are now removed. "Furthermore, all connections are clearly laid out in the viewing area in front of the user. They can be easily visually checked and also repaired, if necessary," says Bernsmann. The base of the DEXTech™ measures only 30 x 57 cm, such that several devices can be placed side by side without any problems whereby the throughput can be further increased.

The system could already impress with its performance at the interlaboratory testing (LVU) "Feed Fat 2013" of the European Reference Laboratory EURL in Freiburg. All analytical results came very close to the consensus values, which were determined by the EURL for LVU from data of all participating laboratories. "The future development of the DEXTech™ system will be aimed at a further reduction of the time needed for sample clean-up and thereby even more solvents will be saved," explains Bernsmann. "In addition, we would like to work on the reduction of materials whilst maintaining the same performance."

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