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Automated recognition of target compounds at low levels in environmental samples by means of capillary gas chromatography–mass spectrometry with dedicated mass spectral libraries and the macro program AUTARG

I. Description of the macro program AUTARG ☆

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ABSTRACT

Automatic pesticide screening was performed using a macro program to compare mass spectra acquired during GC–MS with those in designated mass spectral libraries containing a limited number of target compounds. The automated evaluation procedure enabled fast recognition of pesticides in complex chromatograms. The application of reconstructed ion chromatograms of two characteristic ions and time window programming even leads to the recognition of target compounds overlapped by matrix substances and background noise.

INTRODUCTION

In environmental analysis, capillary gas–liquid chromatography (GLC) plays a major role in the detection of pollutants or pesticide residues in soil or water samples. Many hundreds of such contaminants can be directly analysed by GLC, a great many others after derivatization. These large numbers of environmental pollutants have to be separated from each other and from matrix compounds. Screening analysis has been successfully performed using gas chromatography with capillary columns, and simultaneous detection with two different selective detectors, usually combinations of electron-capture detection (ECD), nitrogen–phosphorus detection and flame photometric detection. Although these detectors are highly selective, matrix compounds that survive the clean-up procedures may also produce signals. As a consequence, the chromatograms generated often appear very complex.

Therefore, in recent years, the mass spec-

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☆ The macro program AUTARG can be obtained from Professor Dr. H.-J. Stan, Institute of Food Chemistry, Technical University of Berlin, Gustav-Meyer-Allee 25, D-13355 Berlin, Germany. A description is included.

trometer, which has been used in confirmatory analysis for decades in the field of environmental analysis, has also gained popularity in screening analysis. This new application may become a domain of the mass selective detectors. In the course of the investigation of a series of ground-water samples, we found that most of the chromatograms obtained by GLC with ECD could not be evaluated satisfactorily because of the large number of matrix peaks. Screening analysis with the HP 5970 mass-selective detector with cyclic scanning and searching in a designated mass spectral pesticide library showed surprisingly good results. Manual data evaluation still remained very time-consuming, although the search was done only in a designated library. Therefore, the macro program AUTOEVAL was developed, which simulated automatically all the steps usually applied in manual data evaluation [1,2]. The program has been used successfully for about the last 2 years for pesticide residue analysis in food and various kinds of environmental analyses in ground- and surface water as well as soil samples.

In this paper we describe an updated version of this program, now called AUTARG, which stands for automated target analysis.

EXPERIMENTAL

Sample preparation and the experimental conditions of GC–MS are described in Part II of the paper [3].

HARDWARE AND SOFTWARE REQUIREMENTS

After installation the program AUTARG runs as a part of the HP ChemStation software for MS-DOS. Therefore, hardware requirements are the same as for the HP ChemStation software (personal computer with a 386 processor, hard disk and at least 4 MB of RAM). The use of a math coprocessor is recommended to speed up data analysis.

We run the HP ChemStation on a 486 PC with 8 MB of RAM, a 120-MB hard disk and MS-DOS 5.0 and Windows 3.1 as the operating system.

DESIGN OF THE PROGRAM AUTARG

When environmental samples are analysed by GC–MS in full-scan mode, all eluting substances produce signals. The resulting total-ion chromatograms (TICs) are, therefore, often of a very complex nature. One of the merits of mass spectrometric detection is, however, that information about substances is highly specific and can be matched with mass spectral libraries. In order to obtain the best search results, most of the spectra measured require background subtraction to reduce interfering signals. Manipulation and library search are aided by processing functions offered by the operating software.

The aim, which was successfully achieved with the macro program AUTOEVAL, was the automated performance of all the steps necessary for manual data evaluation. The new program AUTARG, taking full advantage of Windows, follows the same line and consists of five program parts that can be integrated into the data analysis window of the HP ChemStation software. They provide the following functions.

AUTARG Level 1 performs an automated library search of every automatically integrated peak against one or two user-defined libraries. It is written in HP ChemStation macro language.

AUTARG Level 2 uses reconstruced ion chromatograms (RICs) to search for compounds at very low concentrations. It is also useful in detecting peaks that are overlapped by matrix compounds. It is written in HP ChemStation macro language.

AUTARG Set-up is used to define user parameters, *e.g.* the names of the mass spectral libraries for automatic searching, thresholds, time windows and so on. It too is written in HP ChemStation macro language.

AUTARG Control-file editor is a program to build up the so-called control-files needed for the AUTARG Level 2 search. It is written in Visual Basic for Windows.

AUTARG Results is a program for viewing, editing and printing the results obtained from Level 1 and Level 2 searches. It is written in Visual basic for Windows.

Fig. 1 shows the data analysis screen with the opened pull-down menu of AUTARG.

Fig. 1. Data analysis screen with the opened pull-down menu of AUTARG.

Description of Level 1

A click on menu point Level 1 starts the integration of the TICs according to user-defined integration parameters. The user can choose, for example, to use autointegration or a special events file. After integration, every integrated peak is background subtracted by subtracting successively the mass spectra at peak start and peak end and then compared with one or two target compound libraries. It is also possible to specify a hit quality threshold, so that Level 1 only switches into the second library if this threshold is not met. By testing various conditions, we found that library searches on small peaks may give poor results if background subtraction has been performed. Therefore, Level 1 also performs a library search without background subtraction for each peak and checks whether the hit quality is higher with or without background subtraction. The results, compiled in three different result tables together with a picture of the TIC, can easily be accessed by

choosing Results from the AUTARG menu, as shown in Fig. 2.

The first results table, called AUTARG Results Level 1, contains the complete analysis report of AUTARG Level 1, including the sample description (sample name, corresponding data file, acquisition date, method file, operator) as well as the names of the target compound libraries. All integrated peaks are listed with their retention times and search results. If a library search is successful, the three best matches of each library search are reported with their substance name, hit quality and reference number in the corresponding library. If the user has also specified a retention time reference standard (*e.g.* aldrin), the expected retention time for each compound found by library search is calculated. Peaks with no search results are reported as such, with only their retention times.

In a second results table, called Important Peak List, only those results of the library search that have met user-defined thresholds for high
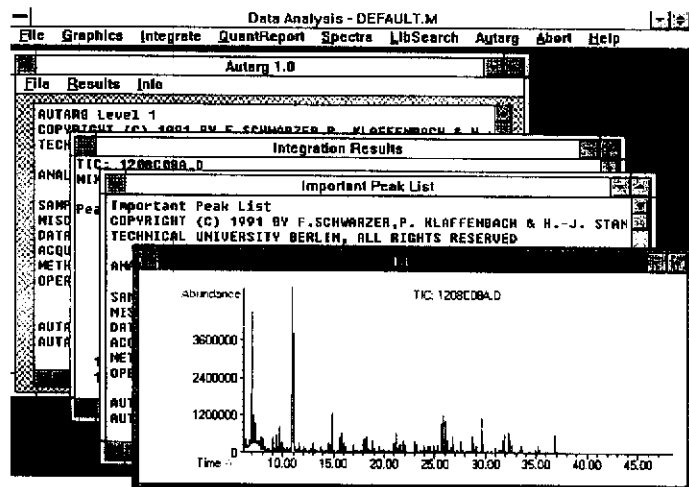

Fig. 2. Results of a AUTARG Level 1 search.

quality and retention time windows are compiled. For example, this list might contain only search results with a hit quality better than 80 and also all peaks with a high quality less than 80 if exhibiting a difference in retention time smaller than 1 min from that laid down in the library for the suggested compound.

This also enables the recognition of target compounds overlapped by matrix components, which produce poor library search results, and emphasizes the retention time as a very important independent piece of information. On the other hand, any similarity between the mass spectrum of the sample peak and the reference spectrum must be considered as purely coincidental if the retention times of the reference target compound and sample peak are significantly different.

The third results table contains the integration results of all peaks with all details such as peak area, peak width, resolution, peak start and peak end. Together with the TIC picture, the integration results table presents an overview of all compounds detected in the sample.

Compared with AUTOEVAL, AUTARG Level 1 offers the following advantages. With AUTOEVAL, all results were directly sent to the printer. This turned out to be very time-consuming when using AUTOEVAL as a part of a method because of the low speed of the printing process. AUTARG Level 1, therefore, prints all results to files, which speeds up the data analysis process considerably. Data processing now consumes less time than needed by the GC–MS system to cool down after a run with temperature programme and to equilibrate before the next start. A second advantage of AUTARG Level 1 is that the analyst can check the results first on the screen and can decide later what to print out. This saves printing costs and reduces paper consumption enormously. Finally, the automatic comparison of search results with and without background subtraction always guarantees the best search results.

Description of Level 2

As mentioned above, the AUTARG Level 1 results table may contain some search results with a poor hit quality but excellent correlation between the retention times for the peak searched and the suggested library compound. In this case, the target compound may be overlapped by a co-eluate from the matrix and manual evaluation must, therefore, be performed. Such a manual verification procedure, performed to confirm the identity of a compound generating a peak overlapped by the peak of a matrix compound, is demonstrated below.

In a groundwater sample AUTARG Level 1 found 2-(4)-chlorophenoxy-2-methyl-propionic acid pentafluorobenzyl (PFB) ester with a hit quality of only 44 but an excellent match in retention time. Fig. 3 shows the TICs of the groundwater sample and a zoomed view of the peak under investigation.

The mass spectrum taken at the apex and the library search result are shown in Fig. 4. The base peak in the spectra of the unknown is ion m/z 149, which commonly belongs to plasticizers of the phthalic ester group. Visual comparison of the spectrum of the unknown and the spectrum of the reference compound from the library shows that the appropriate ions for 2-(4)-chloro-phenoxy-2-methyl-propionic acid PFB ester also appear in the spectrum of the unknown.

For further confirmation of the hypothesis of two compounds overlapping in that one peak,
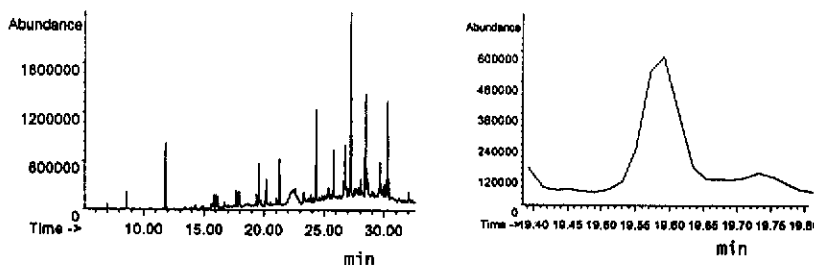

Fig. 3. TIC of a ground water sample. Left: Complete chromatogram. Right: Portion under investigation enlarged.
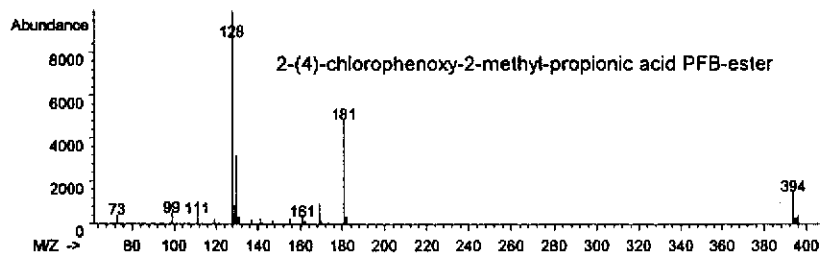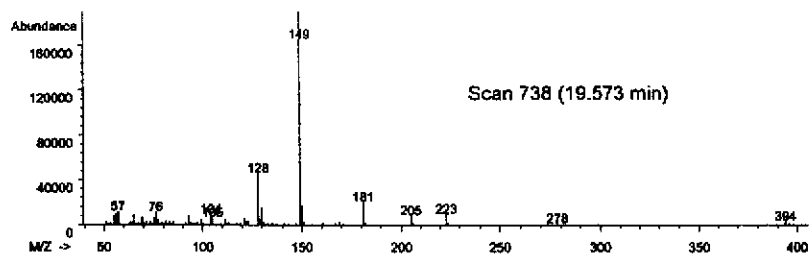
Fig. 4. Mass spectrum of the "unknown" (top). Reference spectrum from the library (bottom).

the use of reconstructed ion chromatograms (RICs) proved to be helpful. The RICs of the two selected specific ions of 2-(4)-chloro-phenoxy-2-methyl-propionic acid PFB ester, m/z 128 and m/z 394, and m/z 149, which is suspected to originate from the co-eluting phthalic ester, are shown in Fig. 5.

It is evident that the traces of the target compound ions and that of the ion suspected to belong to the plasticizers show that the two compounds do not elute exactly at the same time. In order to obtain a target compound spectrum of maximum purity, a scan was taken at that time at which the plasticizers (ion m/z 149) have almost completely eluted. After background subtraction, the library search reported

the recognition of 2-(4)-chlorophenoxy-2-methyl propionic acid PFB ester with a hit quality of 99.

As shown in the example, manual evaluation of peaks exhibiting poor hit quality to suggested library compounds but a good correlation in retention time can provide excellent results when using RICs of appropriate selected ions. A disadvantage of this technique is that it is very time-consuming when applied to a large number of peaks. Therefore, we developed AUTARG Level 2 to execute automatically all the steps described above. The only task the user has to perform is to create so-called control-files for AUTARG Level 2. This can easily be done with the Control-file editor, of which a screenprint is shown in Fig. 6.
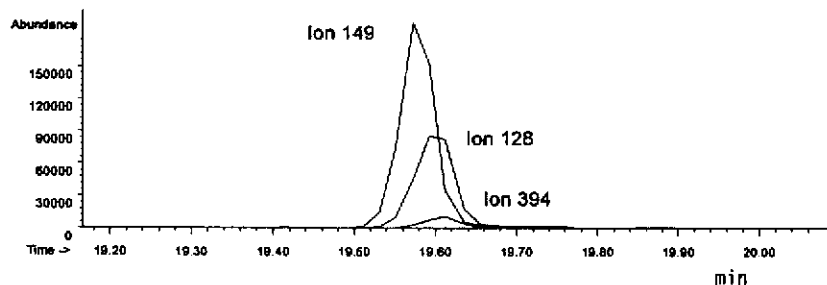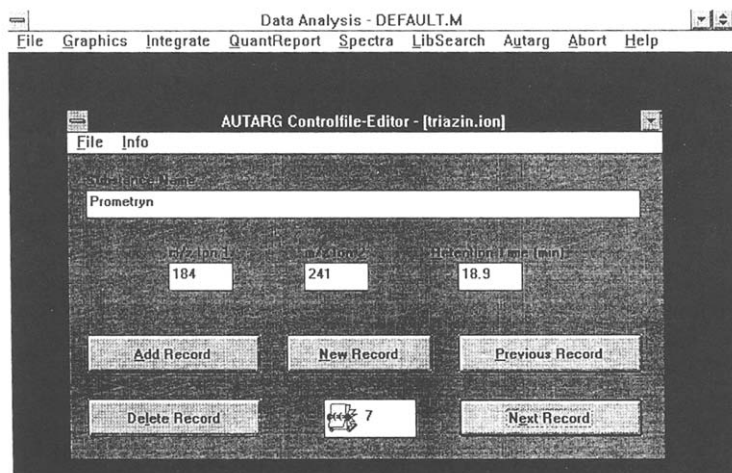

Fig. 5. RIC of the "unknown".

Fig. 6. Control-file editor for AUTARG Level 2.

For each substance, AUTARG Level 2 needs the target compound name, the two selected specific ions and the retention time expected for the target compound. A group of target compounds can be combined in one control file. For example, all triazine herbicides can be compiled in a control file named triazin.ion, which we use in our laboratory, as shown in Fig. 6. One control file can hold up to 100 target compounds.

When starting the Level 2 search from the AUTARG menu, Level 2 asks for the number of control files that should be used for search. Up to five such control files are allowed for one search run. Having started by simply marking the control file names on the corresponding file list, Level 2 begins to check for the first target compound in the first control file. This is done by redrawing the specified ion traces in a user-defined time window, usually 1 min, around the expected retention time of the target compound. Only if Level 2 has found peaks in the traces of both ions will it proceed to check whether peaks appear at the same retention time in both of the ion traces. If their difference in retention is smaller than 0.015 min, Level 2 assumes that the two ions originate from the same target compound and continues by taking a scan at the apex of the peak followed by background subtraction. This is performed by subtracting the scans taken at peak start and peak end. The resulting spectrum is then searched against a target compound library. When looking at the example illustrated in Fig. 5, it can be seen that the apex of the target compound ion traces does not fit exactly to the apex of the matrix compound peak. Therefore, the spectrum of the scan selected by Level 2 shows already a good correspondence to that of the target compound sought. Further background subtraction at peak start and peak end of the target compound eliminates the interfering ion m/z 149 almost completely, so that the library search now results in a hit quality of 99. In other cases, usually when the peak is very small, it may be preferable not to subtract any background from the current spectrum. Therefore library search is also performed without background subtraction. Only the better result is reported.

Level 2 then continues to search for the next target compound listed in the control file. When no more compounds are found in the current control file, Level 2 switches to the next control file. As mentioned above, a maximum number of five control files can be used for one Level 2 run with a total search capacity of up to 500 target compounds.

All positive search results are compiled in the AUTARG results Level 2 table with a complete sample description (sample name, corresponding data file, acquisition date, method file, operator), the names of the control files and the library used. Each peak with a positive search
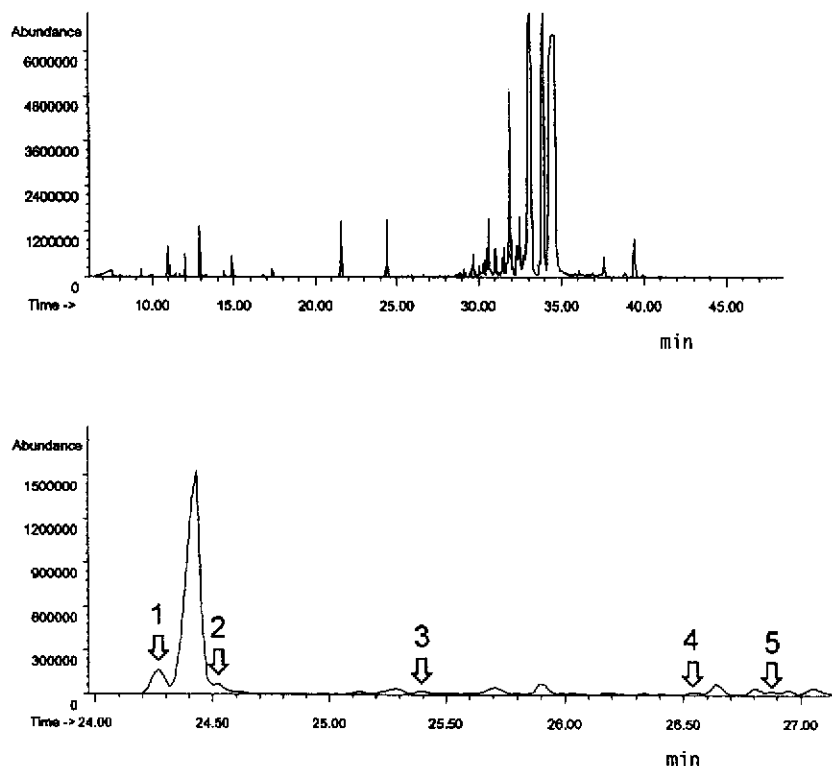
Fig. 7. TIC of groundwater sample. Top: Complete chromatogram. Bottom: Zoomed part with the peaks labeled which were found by AUTARG Level 2.

result is described by the scan number, the retention time at which the spectrum was taken and the three best hits.

A particular feature of Level 2 is also based on the RIC technique. Level 2 can find such peaks that are normally overlooked, being hidden in the noise. These peaks naturally are not integrated by AUTARG Level 1, or by any integration software, and as a consequence are not searched. Since the signal-to-noise ratio is, with most of the ion traces, orders of magnitudes better than with TIC, the presence of compounds can be spotted at very low concentration levels depending, the course, on the overall abundance of the fragment ions selected. Hence, we developed AUTARG Level 2 not only to support the recognition of a target compound overlapped by matrix compounds, but as a general supplement for AUTARG Level 1 in order to reduce the determination limit. This is demonstrated with the example below. Fig. 7

shows the TIC of a groundwater sample for which Level 1 reported no search results. Level 2 was therefore started, with a control file for phenoxy acid herbicides for further search, resulting in the recognition of five target compounds as listed in Table I.

The peaks were labelled with arrows in the zoomed part of the TIC.

Searching for target compounds in parts of a TIC where no analyst would suspect the presence of any target compounds, because no peaks can be seen (e.g. peaks 3, 4 and 5), makes Level 2 a very valuable tool. The reason why such peaks, which are easily overlooked in the TIC, can be recognized is the high signal-to-noise ratio in the traces of discrete target compound ions. In Fig. 8, as one example, the RIC traces of two ions of dichlorpropionic acid PFB ester (peak 5) are shown.

With a scan taken at the apex of the peak (Fig. 9, top) a library search was performed, which

TABLE I

TARGET PESTICIDES RECOGNIZED WITH AUTARG LEVEL 2 APPLYING A CONTROL FILE FOR PHENOXY ACID HERBICIDE PFB ESTERS

MCPA = 4-Chloro-*o*-tolyloxyacetic acid.

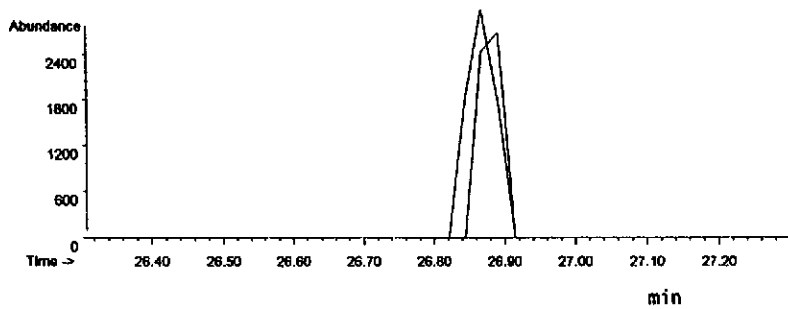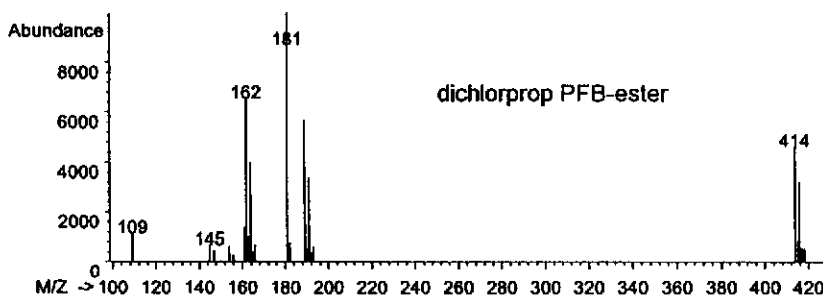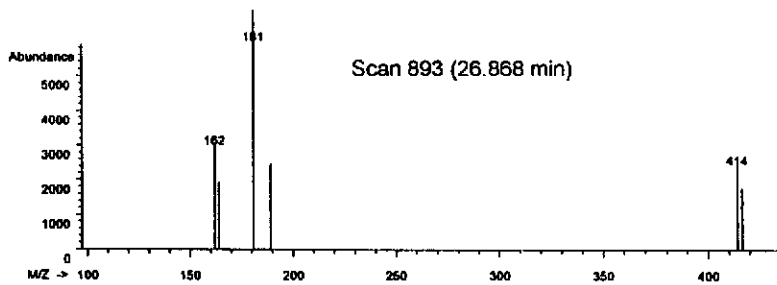| Peak	Target compound	Hit quality
1	2-(4)-Chlorophenoxy-2-methyl-propionic-acid PFB ester	98
2	2,4-Dichlorobenzoic acid PFB ester	96
3	Mecoprop PFB ester	83
4	MCPA PFB ester	42
5	Dichlorprop PFB ester	52

Fig. 8. RIC of two ions of dichlorprop-PFB-ester (m/z 414 and 162).

Fig. 9. Scan taken at the apex of the ion traces (top). Result of library search (bottom).

resulted in a hit quality of 53 for dichlor-propionic acid PFB ester (Fig. 9, bottom).

A visual inspection of the two mass spectra confirms their correspondence in the indicative ions.

CONCLUSIONS

Automated evaluation of GC–MS data from complex total ion chromatograms of environmental samples can be performed easily with the AUTARG program. The evaluation is executed in the short time usually necessary to re-equilibrate the GC oven for the next temperature-programmed analysis. The quality of the evaluation is constant throughout the analysis, in contrast to that of manual data evaluation. The use of the macro in combination with designated libraries containing only a limited number of target compounds leads to reliable results that can be verified very quickly by the analyst.

The application of reconstructed ion chromatograms of two characteristic ions with time window programming enables the recognition of target compounds overlapped by co-eluting matrix compounds. The method is also valid for target compounds at very low concentration levels, which produce peaks in the total ion chromatogram not exceeding the noise level.

REFERENCES

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