Implementation of Deconvolution Reporting Software (DRS) in Doping Control

Application Note

Forensic Toxicology

Authors

W. Van Thuyne, P. Van Eenoo,
W. Van Gansbeke, and F. T. Delbeke
Doping Control Laboratory
Ghent University – UGent
Technologiepark 30, B-9052 Zwijnaarde
Belgium

Abstract

The deconvolution reporting software (DRS) combines quantitation and identification based upon self-created libraries and the NIST library. This study shows that the DRS software can be implemented in routine doping control and is very reliable. For the review of full scan data, it was found that one reviewer and DRS can replace the current approach of two reviewers.

Agilent Technologies

Introduction

Several screening methods currently used in doping control rely on GC-MS. Using this technique, stimulants, anabolic androgenic steroids, aromatase inhibitors, narcotics, and other substances can be detected in the urine of athletes [1, 2]. Except for anabolic steroids, which have to be detected at a minimum required performance level (MRPL) of 10 ng/mL according to the World Anti-Doping Agency (WADA) guidelines, these substances can be detected using the GC-MS instrument in the full-scan mode.

At present the GC-MS data generated is interpreted by two independent reviewers by looking at two ion traces of diagnostic ions in a retention time window typical for the component. In case of a suspicious sample, the full-scan spectrum aids in the decision to start a confirmation procedure. This process is time consuming and requires trained and concentrated staff to avoid reporting a false negative result.

The deconvolution reporting software (DRS), introduced in 2004, is a tool to produce an easy-to-read report based upon four different aspects. These are retention time, mass selective detector (MSD) ChemStation software, an automated mass spectral deconvolution and identification system (AMDIS), and the National Institute of Standards and Technology (NIST) library.

The goal of this study was to evaluate the possibilities of DRS in routine screening methods in doping control by implementing it in the GC-MS screening method for narcotics and stimulants.

Materials and Methods

All urine samples were extracted according to the procedure previously described by Van Thuyne et al. [2]. Analysis was carried out on an Agilent 5973 MSD directly coupled to an Agilent 6890 GC. Interpretation of the results was performed by two scientists, after which each sample was processed using the DRS present in the MSD ChemStation software (Revision D.03.00).

The sensitivity of the DRS software was examined by spiking nine different components to negative urine in the range 10 to 500 ng/mL. These substances were: mephentermine, amphetamine, methoxyphenamine, crotethamide, benzylpiperazine, MDEA, methylphenidate, pipradrol, morphine, and fenethylline.

Afterward, 1,366 routine samples were interpreted by both operators, followed by the DRS software.

Results and Discussion

The goal of deconvolution is to extract one signal from a complex mixture. In this way, the mass spectrum of coeluting peaks can be purified, allowing a better identification and confirmation of its structure. The tool to perform this process in the DRS software is AMDIS. AMDIS works by grouping all extracted ions having the same peak apex and a similar rise and fall of the ion trace. AMDIS has no correlation with peak integration.

In order to use the DRS software, several steps have to be taken. The first one is to develop or reform an existing GC method to a retention time-locked method. As the identification is based upon both retention time and mass spectral criteria, it is of utmost importance to keep the retention times constant. After locking the GC method, a library has to be created and calibrated (that is, adding retention time data). The development of the library is done by injecting one or more reference mixtures and can be performed in two different ways. The first one relies on the MSD ChemStation software. Using this procedure, all information present in the spectrum of the component is stored in the library. The second way is to use the deconvoluted spectra obtained by AMDIS to create a library. Both methods have their advantages. Using the ChemStation software is much faster than using AMDIS. However, using this approach, all monitored ions, including those that are part of the background, are present in the reference spectrum. Using the deconvoluted spectrum results in a limited number of ions, which can be attributed with a certainty of 100 percent to the reference component, which are then transferred in the library. Using amphetamine as an example, 333 different *m/z* values were entered in the library using the ChemStation software, while this number was restricted to 12 ions using the AMDIS approach. As a result, analysis of a spiked sample with MDA (500 ng/mL) results in a match factor of only 36 percent using the ChemStation software, while using the AMDIS database resulted in a match factor of 98 percent. Every small ion present in the spectrum in the ChemStation library is taken into account to determine the match factor. The absence of these ions in the deconvoluted spectra (because they are not present or belong to the background and therefore are filtered out) results in the low match factor.

Table 1. DRS Output of MDA Using the ChemStation Library (A) and the AMDIS Library (B)

A							
R.T.	CAS number	Compound name	Agilent ChemStation amount (ng)	AMDIS Match	R.T. Diff sec.	NIST Reverse match	Hit number
8.9707	4764174	MDA-TMS		**36**	–4.0		
B							
R.T.	CAS number	Compound name	Agilent ChemStation amount (ng)	AMDIS Match	R.T. Diff sec.	NIST Reverse match	Hit number
8.9749	4764174	MDA-TMS		**98**	0.0		

After the library is optimized, several options can be selected to customize the layout of the output, including a quantification using the ChemStation software and a reconfirmation of the detected substances using a comparison with the NIST library. However, not all substances are present as their TMS derivatives in the NIST library and not all substances are added in the NIST with their common names. Therefore, the reconfirmation using NIST was skipped from the processing method. However, for unknown samples this can be a helpful tool.

In order to optimize the cutoff match value used by the DRS negative urines samples were spiked with nine different components in a concentration range between 10 and 500 ng/mL. The selection of the components was made based upon their full-scan spectrum. TMS-derivatized morphine shows a good spectrum for deconvolution because it possesses numerous ions over its complete mass spectrum with medium to high relative intensities. The other selected components, however, only have a poor mass spectrum. These substances are stimulants, showing only one abundant fragment ion with a low specificity and a small molecular ion. The goal of selecting these substances was to investigate the potential influence of a (small) matrix interference on the DRS match value obtained. This value should be optimized in such a way that all substances can be detected at a concentration as low as possible without the risk of a false positive result. This was the case when applying a cutoff value of 45 percent at a concentration of 50 ng/mL, where a false positive result for methoxyphenamine was obtained (Table 2).

Setting the cutoff value to 65 percent would result in false negative results for MDEA and morphine. At a concentration of 100 ng/mL, which is below the MRPL of all substances in this screening method, all nine components are detected using a cutoff of 65 percent. Therefore, this value of 65 percent was used. In addition, a maximal deviation in retention time of 6 seconds compared to the reference library was applied. This makes retention time locking of utmost importance.

Table 2. DRS Output of Negative Urine Spiked with Nine Components at 50 ng/mL Using a Cutoff of 45 Percent

R.T.	Compound Name	Agilent ChemStation Amount (ng)	AMDIS Match	R.T. Diff sec.	NIST Reverse match	Hit number
3.6341	Mephentermine		91	–4.1		
4.7247	Amphetamine-TMS		88	–2.3		
8.4520	**Methoxypenamine-TMS**		**45**	**2.9**		
9.2010	Crotethamide		79	–1.7		
9.2239	Benzylpiperazine-TMS		91	–1.5		
10.0936	MDEA-TMS		49	–1.3		
10.5285	Methylphenidate-TMS		73	–1.0		
11.8508	Pipradrol-TMS		79	2.0		
13.4639	Morphine-bis-TMS		56	–1.2		
14.4412	Fenethylline		86	–0.6		

In order to test the capabilities of the DRS, a library containing more than 100 components was created to implement in the screening method for stimulants and narcotic agents. The DRS interpretation of the data was performed after both operators had reviewed the macros of the samples in order to avoid influence of the DRS output on the operators' conclusion. The DRS did not produce any false negative results.

All positive results observed by the operators were also picked out by the DRS. In total, 105 samples contained one or more substances identified in both ways. However, the DRS picked out three additional positive samples, which remained unnoticed by the analysts. The first case was a sample positive for amphetamine. Normally amphetamine elutes at a retention time of 4.78 minutes and the ions *m/z* 116 and *m/z* 192 are monitored. Figure 1A shows the ion traces in a QC sample. As can be seen, a systematic interference elutes at the end of this retention time window that can also be observed in a negative urine sample (Figure 1B). With this knowledge, both operators interpreted Figure 1C as negative. However, due to a large interference at the beginning of the chromatogram, retention times were shifted with approximately 0.1 minute. Because the peak apex was found within an interval of 0.1 minute from the expected retention time, the DRS recognized this sample as an amphetamine-positive sample, which was confirmed during a consecutive B-analysis.

The second case was a sample positive for methylphenidate. Figure 2 shows the ion traces as printed in the macro and the corresponding mass spectrum. The lack of the detection of *m/z* 91, diagnostic for methylphenidate, and differences between the obtained spectrum and a reference spectrum lead to the conclusion that this sample was negative. However, DRS identified methylphenidate, which was later confirmed by the detection of ritalinic acid in the diuretic screening method.

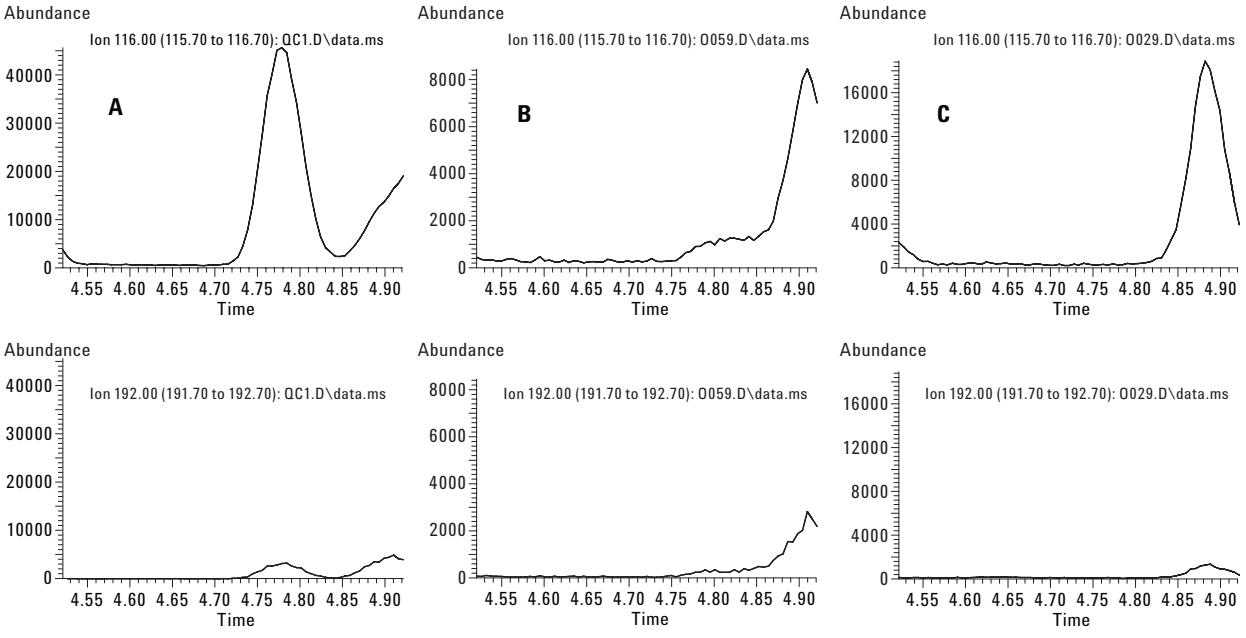

Figure 1. Ion traces for amphetamine in a QC sample (A), negative sample (B), and DRS-positive sample (C).

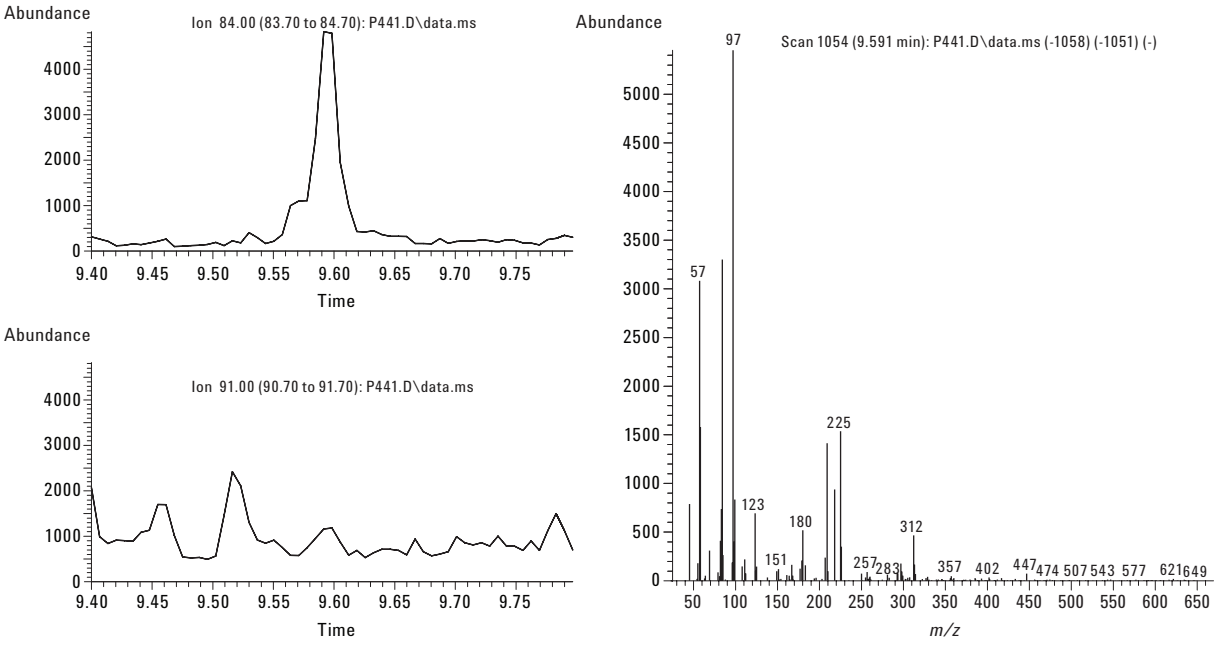

Figure 2. Ion traces for methylphenidate and corresponding spectrum for the peak at RT 9.59 min.

The third sample was positive for morphine. Because of the structural resemblance between morphine and hydromorphone, both substances elute within a small time interval and have the same diagnostic ions monitored in the screening method. At MRPL level, both substances are separated by 0.06 minute as can be seen in Figure 3B.

Obviously, the routine sample shown in Figure 3A contains morphine. A confirmation procedure pointed out that the concentration was higher than 50 µg/mL. According to the DRS software, this sample also contains hydromorphone. Because the ion traces in the macro are scaled to the highest peak detected in a retention time window, small amounts of a substance closely eluting to a highly concentrated peak remain

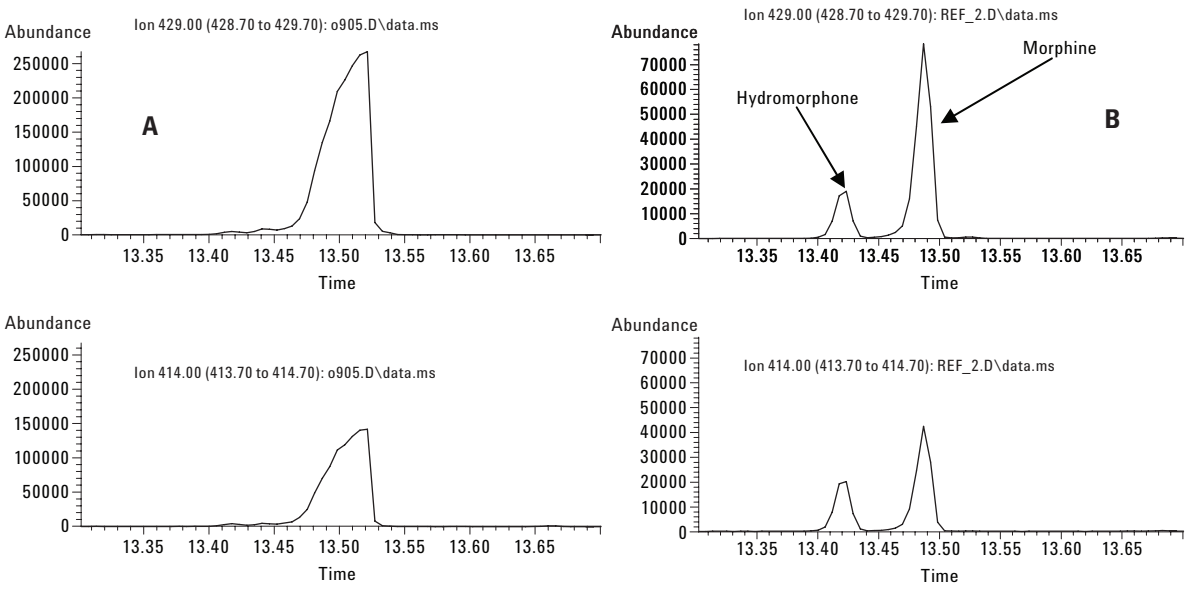

Figure 3. Positive screening result for morphine (A) and QC sample for morphine/hydromorphone (B).

unnoticed. The presence of hydromorphone in this sample could be confirmed. The presence of hydromorphone in the urine of people taking high amounts of morphine has been described in the past [3].

Besides the presence of other doping substances, matrix interferences can also hamper the detection of substances of interest. As described previously, the use of ethyl acetate as extraction solvent for a comprehensive method for the detection of anabolic steroids, narcotic agents, and stimulants was hampered because of a large interference at the retention time of several stimulants, including amphetamine [4] (Figure 4). This interference has been identified as glycerol and can also be detected in smaller amounts using diethyl ether (cfr Figure 1A). Applying the DRS on a sample spiked at 500 ng/mL and using ethyl acetate as extraction solvent results in a match factor of 89 percent, proving the applicability of the DRS to identify target substances in complex matrices.

The deconvolution software is developed to operate in the full-scan mode. However, in doping control, anabolic androgenic steroids have to be detected using selected ion monitoring because of the low level excreted in urine. At present, analytical equipment offers the possibility of combining SIM and scan runs in one analytical run due to improved electronics, allowing a faster data transfer. However, the DRS is designed to operate using only one dataset, making its use in SIM/scan impossible.[1] Its use in a method using only SIM, however, is possible. For this purpose, at least three diagnostic ions have to be selected for each component to avoid numerous false positive results. Because only a selected number of ions are used, the resulting match factors are also higher. This requires different settings compared to a processing method for scan data. This optimization has been performed for approximately 20 different anabolic steroids using a match factor cutoff value of 85 percent. Using this processing method, identification of oral-turinabol in a WADA PT-sample was possible.

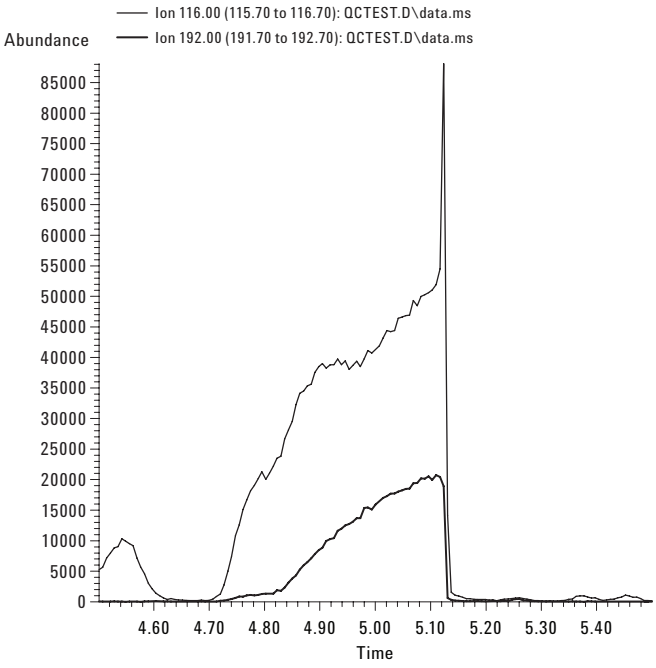

Figure 4. Extracted ion chromatogram for amphetamine using ethyl acetate as extraction solvent.

Conclusions

The DRS software is a tool that combines quantitation and identification based upon self-created libraries and the NIST library. During this study it was proven that DRS software can be implemented in routine doping control and is very reliable. Therefore, the current system of reviewing obtained data, for example, two analysts looking at macros, can be reduced to one person and the DRS software. However, in order to use the DRS software in SIM/scan methodology, several improvements have to be made, including the incorporation of more diagnostic ions for anabolic androgenic steroids monitored in the SIM mode.

Acknowledgements

The authors wish to acknowledge Agilent Technologies for providing the DRS software and the Ministry of Culture, Sport, Youth and Media of the Flemish Community for its financial support.

[1]The DRS software can process both the SIM and scan datafiles from a single SIM/scan run, but requires extra steps. Running the file as acquired will result in the scan data being processed by DRS. The datafile can then be copied to a new name and the Scan file is renamed from DATA.MS to DATASCAN.MS. The SIM file (DATASIM.MS) is then renamed to DATA.MS. The SIM part of the SIM/scan datafile can now be processed with a DRS method constructed for the SIM data.

References

1. C. Ayotte, D. Goudreault, and A. Charlebois, "Testing for Natural and Synthetic Anabolic Agents in Human Urine," *Journal of Chromatography B,* **687**, p. 3–25, 1996
2. W. Van Thuyne, P. Van Eenoo, and F. T. Delbeke, "Comprehensive Screening Method for the Qualitative Detection of Narcotics and Stimulants Using Single Step Derivatisation," *Journal of Chromatography B,* **857**, p. 259–265, 2007
3. E. J. Cone, H. A. Heit, Y. H. Caplan, and D. Gourlay, "Evidence of Morphine Metabolism to Hydromorphone in Pain Patients Chronically Treated with Morphine," *Journal of Analytical Toxicology,* **30**, p. 1–5, 2006
4. W. Van Thuyne, P. Van Eenoo, and F. T. Delbeke, "Possibilities of SIM/Scan Analysis in Doping Control," In: W. Schänzer, H. Geyer, A. Gotzmann, and U. Mareck, (eds.), *Recent Advances in Doping Analysis* (15), Cologne, pp 227–234, 2007

For More Information

For more information on our products and services, visit our Web site at www.agilent.com/chem.

www.agilent.com/chem

For Forensic Use only.

Information is subject to change without notice.

Published in the USA
October 8, 2008
5989-9820EN

Agilent Technologies