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Determination of sulfonamide antibiotics by gas chromatography coupled with atomic emission detection

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Abstract

The paper describes the analysis of nine sulfonamides, chosen as the most widely used representatives of an important class of antibacterial drugs. Atomic emission detection has been found to allow simultaneous quantification and identification of the N^1-methylated derivatives, which are resolved efficiently by conventional capillary gas chromatography. Results are given concerning the linearity of the response and the characterization of the individual compounds by the elemental ratio of their carbon, nitrogen and sulfur content. The method looks promising for the quantitative analysis and confirmation of sulfonamide residues in complex mixtures. © 1998 Elsevier Science B.V.

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1. Introduction

Sulfonamides are currently used in pharmaceutical preparations because of their antibacterial properties. They are extensively utilized in veterinary practice as therapeutic agents and growth promoters raising the risk of the presence of residues that contaminate food products. Such residues are of concern because of their possible carcinogenic activity and the possibility of development of antibiotic resistance in human beings. Therefore, there is a growing demand for analytical methods for the detection, quantitation and identification of sulfonamides in food samples and in a variety of matrices.

Several methods have been proposed for the determination of sulfonamides [1]. Since earlier methods based on the Bratton Marshall colorimetric reaction have become widely adopted, the application of various spectroscopic techniques has been reported [2–8]. Electroanalytical [9,10] and immunochemical [11] detection of sulfonamides and receptor assays [12] have been described recently. Supercritical fluid extraction has been exploited [13,14]. However, most recent analytical procedures rely on the chromatographic separation of sulfonamides effected by thin-layer chromatography, liquid- and gas chromatography, coupled with a selection of detectors of varying sensitivity and specificity [15–30]. Among them, UV detection and mass spectrometric techniques have been most frequently used.

This paper describes the use of atomic emission detection (AED, an element specific detector) [31] for the simultaneous determination of nine sulfonamides, separated by conventional capillary gas chromatography (GC), once converted into their N^1-methyl derivatives. The atomic emission detector is

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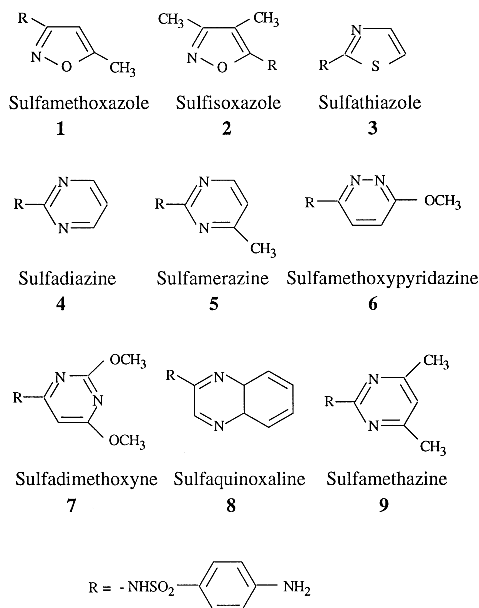
based on a microwave-induced helium plasma where the analytes are transformed into atoms, ions and radicals in different excited states. Upon deactivation, excited states generate photons which are collected using a photodiode array spectrometer, producing an elemental emission spectrum. The intensity of several lines can be registered simultaneously in order to generate a set of various element specific chromatograms. A growing number of applications of GC–AED are reported in the recent literature [32,33]. The selected compounds are listed in Scheme 1.

2. Experimental

2.1. Chemicals

The sulfonamides listed in Scheme 1, solvents (HPLC grade) and diazald (*N*-methyl-*N*-nitroso-*p*-toluenesulfonamide, a precursor to diazomethane) were purchased from Sigma–Aldrich (Milan, Italy).

Research grade gases with stated purity in excess of 99.99 mol % were from Matheson Gas Products (Oevel, Belgium) with the exception of hydrogen which was produced by a Balston 75-31 hydrogen generator.



Scheme 1. Structures of the selected compounds.

2.2. Derivatization

Derivatization via N^1-methylation has been successfully applied to various sulfonamides [30,34]. The N^1-methylation of the selected sulfonamides was performed by a method that uses a 3:1 excess of diazomethane, produced from the reaction of a saturated NaOH solution with an ethanolic solution of diazald [35]. A nitrogen stream carries CH_2N_2 into a solution of sulfonamide dissolved in methanol or acetonitrile. The reaction vessel, which was cooled to the temperature of 0°C during the mixing of the reagents, was then maintained at room temperature, in the dark, for 1 h. The identity of the isolated N^1-methyl derivative was checked by their 70 eV mass spectra recorded with a HP 5982A quadrupolar instrument, on the basis on the known mass spectrometric fragmentation pattern of sulfonamides [36].

2.3. Instrumentation

The GC–AED system (Hewlett-Packard) used is comprised of an HP 5890 gas chromatograph, equipped with a HP 7673A autoinjector, in line with an AED HP 5921A detector, under the control of a HP 5895A chemstation. The AED system features a water cooled quartz discharge tube (a Bennaker cavity) where a microwave induced plasma (MIP) is sustained with helium at atmospheric pressure. Wavelength dispersion is performed by a fixed grating spectrometer with a flat focal plane. Detection is by a moveable photodiode array which allows simultaneous detection of up to four elements. The array range is approximately 25 nm, extending from 170 nm to 780 nm. The selected elements, carbon, sulfur and nitrogen, were monitored at 193.031, 181.354 and 174.261 nm, respectively, using a hydrogen–oxygen mixture as reagent gas. The experimental AED parameters used were as follows: transfer line temperature, 290°C; MIP cavity temperature, 300°C; plasma energy, 70 W; water bath temperature, 60°C; He flow, 130 ml min^{-1}. The reagent gases were admitted at the pressure of 60

p.s.i=414 kPa (hydrogen) and 25 p.s.i.=173 kPa (oxygen).

The operation of the autoinjector included 10 washing cycles with the analyte solution, injection of 1 μl of analyte solution followed by 10 cleaning cycles with methanol. GC separations were obtained on a fused-silica capillary column (12.5 m×0.22 mm I.D.) coated with a 0.33 μm thick film of 5% phenylmethylsilicone bonded phase (HP Ultra 2). The carrier gas was helium, flowing at 1.5 ml min^{-1}, and the injector temperature was 300°C. The temperature profile of the analysis was isothermal at 75°C for 1 min, followed by a temperature ramp of 20°C min^{-1} to 290°C, which was maintained for 5 min.

The performance of AED was compared with that of two different detection systems, keeping constant all parameters of the gas chromatographic separation. A flame ionization detection (FID) system mounted on the HP 5890 gas chromatograph was operated with an air flow of 400 ml min^{-1} and a hydrogen flow of 33 ml min^{-1}. A quadrupolar mass-selective detection (HP 5970 B) system was operated in the scan mode in the *m/z* 60–270 mass range.

2.4. Procedure

Working calibration solutions (containing sulfonamide compounds at concentrations ranging from 3 to 160 μg/ml) were prepared by serial dilution with methanol of appropriate stock solutions. Stock standard solutions (1 mg/ml) were prepared in methanol and were checked for their stability.

3. Results and discussion

The selected sulfonamides eluting from the capillary GC column have been detected by AED. This method allows the simultaneous measurement of several elements, yielding element specific chromatograms. Different analytes are expected to display peak areas proportional to the number of atoms of the specific element reaching the MIP cavity. The detection of C, S and N is allowed within the same chromatographic run by the use of the same reagent gases. Overall, they represent 75% to 85% of the mass of sulfonamides, usually differing in the number of atoms of each element present. The selected emission wavelengths for the three elements are characterised by high sensitivity and selectivity [31]. The detection of the remaining elements, H and O, would require two more chromatographic runs since their emission wavelength are too widely apart in the focal plane (at 656.3 and 777.2 nm, respectively) and different reagent gases are needed. Fig. 1 is an example of a typical GC–AED chromatogram of a mixture of sulfonamides, showing the traces of the response to C, S and N.

3.1. Linearity

The study of the dynamic range, where the analytical response is linear, sets the limits of the sample concentration where the response of the detector yields a reliable quantitative determination. The linearity of the analytical response of the selected elements obtained from various sulfonamides is summarised in Table 1. As clearly shown by the plots of Fig. 2 which illustrate the response of

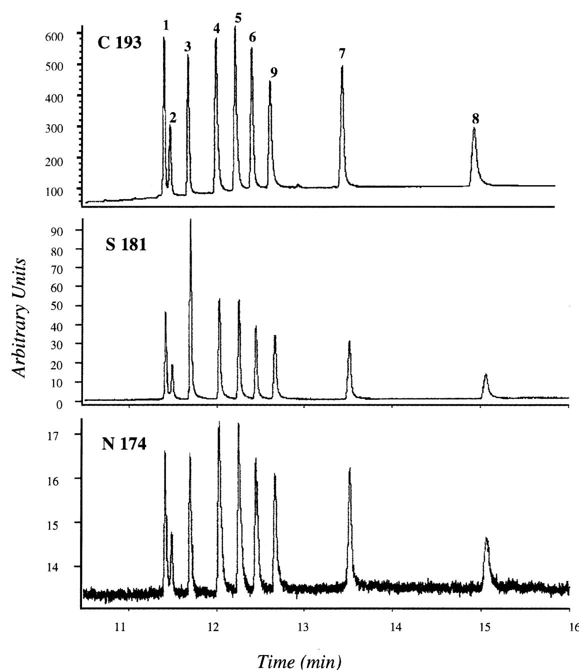


Fig. 1. GC–AED chromatogram of a mixture of N^1-methylated sulfonamides.

Table 1
Parameters of the regression lines of C, N and S measured for selected sulfonamides

N^1-Methylated sulfonamide	Element	Concentration interval	Slope	Correlation coefficient
1	C	3–160 ng	1502	0.992
		3–80 ng	1741	0.993
	S	3–160 ng	563	0.994
		3–80 ng	648	0.995
	N	3–160 ng	33.4	0.996
		3–80 ng	37.1	0.995
2	C	3–160 ng	1128	0.983
		3–80 ng	1454	0.998
	S	3–160 ng	420	0.991
		3–80 ng	504	0.996
	N	5–160 ng	22.1	0.976
		5–60 ng	33.7	0.996
3	C	3–160 ng	1648	0.994
		3–80 ng	1938	0.999
	S	3–160 ng	549	0.991
		3–80 ng	659	0.998
	N	3–160 ng	28.2	0.975
		3–60 ng	42.5	0.998
4	C	3–160 ng	1825	0.984
		3–60 ng	2372	0.995
	S	3–160 ng	724	0.993
		3–80 ng	748	0.992
	N	3–160 ng	38.2	0.982
		3–60 ng	54.1	0.997
5	C	3–160 ng	1841	0.984
		3–80 ng	2317	0.994
	S	3–160 ng	720	0.992
		3–80 ng	854	0.996
	N	3–160 ng	39.9	0.986
		3–60 ng	54.6	0.997
6	C	3–160 ng	2125	0.994
		3–80 ng	2415	0.999
	S	3–160 ng	713	0.994
		3–80 ng	814	0.995
	N	3–160 ng	37.1	0.986
		3–60 ng	51.4	0.999

Table 1. Continued

N^1-Methylated sulfonamide	Element	Concentration interval	Slope	Correlation coefficient
7	C	3–160 ng	2350	0.998
		3–80 ng	2597	0.999
	S	3–160 ng	776	0.992
		3–80 ng	927	0.998
	N	3–160 ng	44.2	0.986
		3–80 ng	55.0	0.995
8	C	3–160 ng	2209	0.993
		3–80 ng	2619	0.999
	S	3–160 ng	586	0.991
		3–80 ng	703	0.996
	N	5–160 ng	37.5	0.962
		5–80 ng	55.2	0.999
9	C	3–160 ng	1611	0.988
		3–80 ng	1730	0.997
	S	3–160 ng	583	0.992
		3–80 ng	688	0.996
	N	3–160 ng	32.9	0.984
		3–60 ng	46.6	0.999

C, S and N from sulfamerazine, excellent linearity is observed in the concentration interval of ca. 3–80 ng/μl of sulfonamide in methanol solution. Deviations are shown above 160 ng/μl, which are more pronounced for N with respect to the other two elements.

It may be observed from the data reported in Table 1 that the linearity of response is not influenced by a different elemental composition of the sulfonamides or by the different sites where the atoms may be placed within the molecule.

The detection limit for each element has been found close to the reported values [31]. Fig. 3 exemplifies the signal-to-noise ratio which is obtained from a sulfonamide sample containing 28 pg of C.

3.2. Qualitative identification of the sulfonamides

An attractive capability of multichannel GC–AED is quantitative element ratioing, yielding information on the empirical formulas of the analytes. In order to obtain a qualitative identification of the sulfonamides, the analytical signals corresponding to C, S and N were recorded and the areas of the corresponding elution peaks were measured. Table 2 summarizes the results obtained for the selected series of sulfonamides, showing the average ratios of elemental response from samples of different concentration, which was varied within the range of ca. 3–80 ng/μl. The fairly constant values obtained within the explored concentration range suggest that the determination of such ratios may represent a reliable aid for the qualitative identification of the analytes. Furthermore, it is confirmed that, within the stated error, similar ratios are obtained from different compounds having the same number of atoms of the selected elements. Incidentally, deviations from the expected values can be taken as indicative of an eluate which is chemically different from the expected sulfonamide or of the presence of an overlapping impurity.

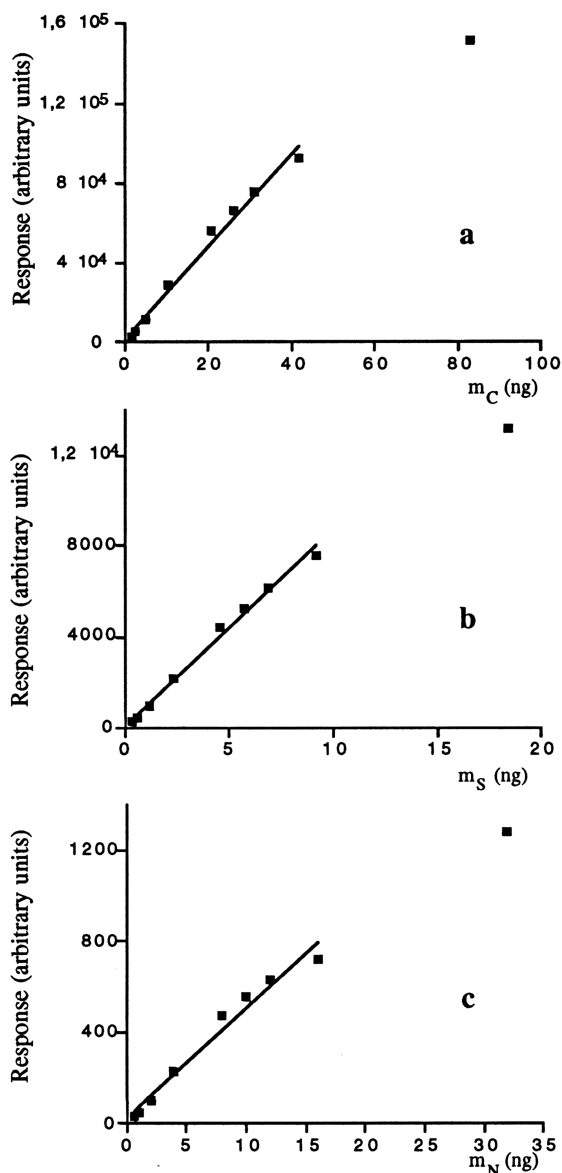


Fig. 2. Calibration plots showing the response of C (a), S (b), and N (c) as a function of the mass (ng) of each element reaching the AED system. The plots are relative to N^1-methylated **5** in the mass range 3–160 ng.

3.3. Qualitative and quantitative determination of sulfonamides using an internal standard

The observed linearity of the AED response within the reported concentration interval allows the quantitative determination of sulfonamides by the use of

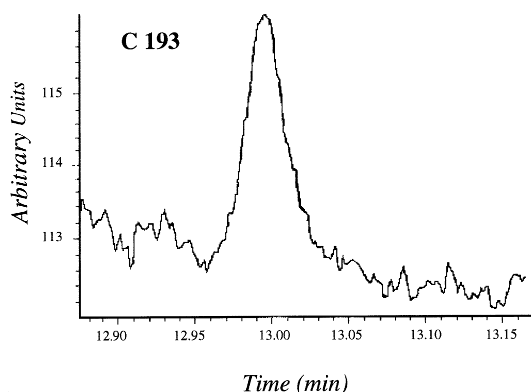


Fig. 3. Chromatographic peak shape arising from AED of an injected sample of N^1-methylated **5** containing 28 pg of carbon.

an internal standard (I.S.). An I.S. was chosen among compounds of similar elemental composition with respect to the sulfonamide analytes. 2-Amino-benzothiazole fulfils this condition, besides being stable under the operative analytical conditions. It does not interfere with the chromatographic separation as its retention time is 7.0 min under the conditions of Fig. 1.

A set of solutions have been prepared for calibration purposes. Each one contained the same amount of standard (80 ng) and the mixture of sulfonamides in individual amounts varying from 5 to 160 ng/μl. The I.S. allows one to gather two pieces of information. In the first place a quantitative evaluation of the sulfonamides present in solution can be obtained from the ratio of the integrated areas of the C, S and N elution peaks for the analyte and the I.S. Eq. (1) can be used to obtain the mass of sulfonamide (m_{SA}) from the integrated areas of the C, S and N signal corresponding to the sulfonamide ($A_{\mathrm{C}}^{\mathrm{SA}}$, $A_{\mathrm{S}}^{\mathrm{SA}}$, $A_{\mathrm{N}}^{\mathrm{SA}}$) and to the I.S. ($A_{\mathrm{C}}^{\mathrm{I.S.}}$, $A_{\mathrm{S}}^{\mathrm{I.S.}}$, $A_{\mathrm{N}}^{\mathrm{I.S.}}$), the total mass ($x_{\mathrm{I.S.}}$) of the elements C, S and N in the internal standard and the weight percentage (y) of C, S and N in the sulfonamide analyte.

$$m_{\mathrm{SA}} = \frac{\left(A_{\mathrm{C}}^{\mathrm{SA}} + A_{\mathrm{S}}^{\mathrm{SA}} + A_{\mathrm{N}}^{\mathrm{SA}}\right)}{\left(A_{\mathrm{C}}^{\mathrm{I.S.}} + A_{\mathrm{S}}^{\mathrm{I.S.}} + A_{\mathrm{N}}^{\mathrm{I.S.}}\right)} \cdot x_{\mathrm{I.S.}} \cdot \frac{100}{y} \qquad (1)$$

The equation was found to hold for all the calibration solutions that were examined.

In the second place, the I.S. may yield information on the elemental ratios within the analytes. To this

Table 2
Relative AED response of C, S and N for selected N^1-methylated sulfonamides

N^1-Methylated sulfonamide	Number of atoms			Relative response		
	C	S	N	C/N	C/S	S/N
1	11	1	3	143.3±8.3	11.2±0.7	12.5±0.9
2	12	1	3	167.0±10.3	11.9±1.3	12.4±1.4
3	10	2	3	131.9±1.7	5.2±0.3	26.1±2.2
4	11	1	4	110.1±5.9	11.2±0.7	9.8±0.7
5	12	1	4	117.1±8.6	12.0±0.8	9.7±0.4
6	12	1	4	118.6±8.7	12.3±1.4	9.7±0.8
7	13	1	4	132.0±2.8	13.5±0.2	9.7±0.3
8	15	1	4	163.0±12.7	17.8±3.2	9.1±1.8
9	13	1	4	128.8±10.7	13.1±0.6	9.8±0.7

end, one can rely on the constancy of the factors of proportionality between the elemental responses, irrespective of the chemical identity of the individual compound under examination [37]. This constant relationship is stated in Eqs. (2) and (3), referring to the response of S and N with respect to C [K(S:C) and K(N:C), respectively].

$$K_{\mathrm{I.S.}}(\mathrm{S{:}C}) = K_{\mathrm{SA}}(\mathrm{S{:}C}) = \frac{A_{\mathrm{S}} n_{\mathrm{C}}}{A_{\mathrm{C}} n_{\mathrm{S}}} \tag{2}$$

$$K_{\mathrm{I.S.}}(\mathrm{N{:}C}) = K_{\mathrm{SA}}(\mathrm{N{:}C}) = \frac{A_{\mathrm{N}} n_{\mathrm{C}}}{A_{\mathrm{C}} n_{\mathrm{N}}} \tag{3}$$

where A_{N}, A_{C}, A_{S} = integrated areas of chromatographic signal (N, C and S profile, respectively), n_{N}, n_{C}, n_{S} = number of atoms (N, C and S, respectively).

The response factors were thus found to be equal within experimental error, when they were calculated from 2-aminobenzothiazole [$K_{\mathrm{I.S.}}$(S:C) and $K_{\mathrm{I.S.}}$(N:C)] or from any of the N^1-methyl derivatives of the sulfonamides **1–9** [K_{AS}(S:C) and K_{AS}(N:C)]. The number of S atoms in any sulfonamide may then be calculated from Eq. (4).

$$n_{\mathrm{S}} = K(\mathrm{S{:}C}) \frac{n_{\mathrm{C}} A_{\mathrm{S}}}{A_{\mathrm{C}}} \tag{4}$$

A similar equation holds for n_{N} which can be obtained from the factor of proportionality K(N:C) and the areas of the elution peaks of the sulfonamide analyte in the N and C chromatograms, once the number of C atoms in the sulfonamide molecule is known. If this is not the case, one may try tentative values until integer numbers are obtained for the number of S and N atoms. Table 3 summarizes the data obtained from the calibration solution containing 40 ng of each sulfonamide together with 80 ng of I.S. There appears to be good agreement between the theoretical number of S and N atoms and the experimentally derived values, allowing the characterization of the individual sulfonamide.

Table 3
Calculation of the number of N and S atoms in the sulfonamide analytes

N^1-Methylated sulfonamide	Theoretical number of atoms			Experimental number	
	C	S	N	S	N
1	11	1	3	1.02	3.21
2	12	1	3	0.99	3.02
3	10	2	3	2.20	3.30
4	11	1	4	1.08	4.08
5	12	1	4	1.08	4.21
6	12	1	4	0.92	3.84
7	13	1	4	0.94	3.87
8	15	1	4	1.12	3.90
9	13	1	4	1.00	4.13

Table 4
Parameters of the regression lines for GC–FID analysis of selected sulfonamides in the mass range 3–160 ng

N^1-Methylated sulfonamide	Slope	Intercept	Correlation coefficient
1	0.730	−0.029	0.9995
2	0.455	−0.029	0.9992
3	0.606	−0.013	0.9996
4	0.889	−0.025	0.9995
5	0.958	−0.022	0.9996
6	0.733	−0.018	0.9997
7	0.815	−0.015	0.9998
8	0.962	−0.026	0.9996
9	0.764	−0.018	0.9993

3.4. Comparison with other GC detections systems

Comparative analyses have been performed with FID and MS detection systems, using otherwise equal gas chromatographic conditions. Table 4 summarizes the GC–FID data, showing the perfect linearity of response within the explored concentration range. However, FID does not yield any information on the chemical identity of the analyte, other than that based on its retention time.

GC–MS offers the major advantage of the qualitative identification of the analytes by their mass spectrum [36]. However the quantitative determination of sulfonamides is hampered by their non-linear response, with deviations depending on the specific compound. This is exemplified by the non-linear behaviour of the response of N^1-methyl-sulfamerazine shown in the plot of Fig. 4.

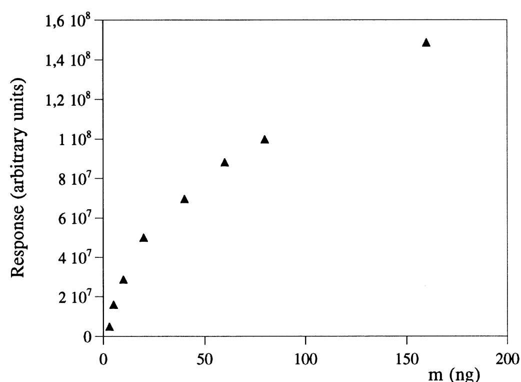


Fig. 4. Calibration plot showing the GC–MS response of N^1-methylated **5** in the mass range 3–160 ng.

In conclusion, the use of AED as a GC detection method following the GC resolution of complex mixtures of N^1-methylated sulfonamides presents the noticeable advantage of combining the qualitative identification of the analyte by the evaluation of elemental ratios with the quantitative determination allowed by a significantly extended linear dynamic range and a low detection limit. Therefore, it may be applied fruitfully to the analysis of sulfonamides (and their metabolites) in biological matrixes, following established extraction procedures [1,27,28,30].

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