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Development and validation of a solid-phase extraction method coupled to high-performance liquid chromatography with ultraviolet-diode array detection for the determination of sulfonylurea herbicide residues in bovine milk samples

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ABSTRACT

This study proposes a fast, simple and sensitive liquid chromatography diode array detector (LC/UV–DAD)-based method for the simultaneous determination of eight sulfonylurea herbicides (bensulfuron methyl, chlorsulfuron, metsulfuron methyl, primisulfuron methyl, rimsulfuron, thifensulfuron methyl, triasulfuron and tribenuron methyl) in bovine whole milk at concentrations lower than the default limit of 0.01 mg kg^{-1} allowed by current legislation (Regulation EC/396/2005 and following Annexes). An effective one-step solid phase extraction (SPE) and clean up procedure was defined with use of Chem Elut cartridges, providing good recoveries for all the analytes tested and with no matrix effects affecting method accuracy. Separation of herbicides was obtained on a C_{18} column by acetonitrile- water gradient elution. Method validation has been performed according to European Commission Decision 2002/657/EC criteria, in terms of linearity, recovery, precision, specificity, decision limit (CC_α) and detection capability (CC_β). Typical recoveries ranged between 78.4% and 99.7%, at the maximum residue limits (MRLs) levels established by Regulation EC/396/2005, with relative standard deviations (RSD) no larger than 10%.

1. Introduction

The wide use in agriculture of herbicides to control a great variety of weeds and grasses, as well as their environmental impact, is a particularly challenging problem. Herbicides represent about 50% of the demand for agricultural chemicals; their prolonged use represents not only an environmental risk but also a health hazard for their retention in crops. The intensive application of herbicides has resulted in the contamination of the atmosphere, ground and waste waters, agricultural products and, consequently, in the direct or indirect pollution of food and food products and biological systems. Milk-producing animals, such as cows, may accumulate residues of these pesticides through carry over processes from contaminated feed, grass and corn silage, water, top-layer soil and inhaled air. As a result, residues of some harmful pesticides can be detected in raw bovine milk samples [1,2].

Although, according to good agricultural practices, the more recent herbicide formulations are designed to offer advantages of the highest selectivity together with the lowest persistence in the environment, monitoring pesticide residues in foodstuff is very important for the assessment of their harmful effects to humans [3,4].

In the European countries, the safety of milk for consumers is ensured by the European Union and the Government agencies, that have laid down maximum residue limits (MRLs) for hundred of pesticides and particularly for several herbicides [5–9]. Regulation (EC) No 396/2005 [6] and the new rules in force since 1 September 2008 [8,9] establish a Community regime for setting and controlling pesticide maximum residue levels in food and feeding stuffs. This European Union legislation harmonizes pesticides MRLs and fixes for these substances a MRL default value at 0.01 mg kg^{-1} [10].

Sulfonylureas (SUs) are low application rate (10–40 g/ha) herbicides, introduced in integrated pest management programs for pre-emergence or early post-emergence control of many grasses and most broad-leafed weed species in crop protection. They have replaced the old high-application-rate herbicides because of their low toxicity to mammals and rapid degradation in soil and water [11]. Therefore, the concentrations of these herbicides usually found in the environment are very low (ppt or ppb range) [12].

Liquid chromatography (LC) is the preferred approach for monitoring these polar and thermally labile herbicides [13]. Most of the known applications are based on high performance liquid chromatography (HPLC) using reversed phase columns, followed either by conventional ultraviolet (UV) detection or diode array

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detection (DAD), for their molar absorptivity in the UV region, or mass spectrometric (MS) detection, interfacing different ionization techniques [14–17]. However, it is well known that the HPLC/MS instrumentation is fairly expensive and not always available in all environmental laboratories, especially if they have limited financial resources. Therefore, sufficiently selective and sensitive analytical methods based on inexpensive instrumentation, such as HPLC with DAD detection, may be highly desirable for routine monitoring pesticide residues. Stoev and Stoyanov [18] demonstrated that the reliability of identification by DAD is comparable to that of low resolution MS. In fact, the enlarged wavelength scanning range (200–900 nm) of the modern DAD and its increased sensitivity increased the reproducibility of the UV–vis spectra. Therefore, the analysis of pesticide residues by LC–DAD may be proposed as alternative to LC–MS analysis. In addition, an adequate and effective cleanup of milk samples, should be involved before HPLC trace analysis, in order to reach the concentration level requested, with no matrix interferences.

The determination of pesticide residues in milk has presented problems because the most common approach has involved total extraction of fat together.

In recent years, several extraction methods have been proposed for the analysis of phenylurea, triazine, fenoxy and benzoylurea herbicides [19–24], organophosphorus, organochlorine and pyrethroid pesticides [25–27], neonicotinoid insecticides [28] and for the multiresidue analysis of pesticides [29] in milk samples, based on solid-phase extraction (SPE) [19–21,25,26,28] hollow fiber membrane-protected solid-phase microextraction (HFM-SPME) [22], dispersive solid-phase extraction (DSPE) [30], pressurized liquid extraction (PLE) [24,27], as alternative to classic liquid–liquid extraction.

Residues of metsulfuron, chlorsulfuron and bensulfuron in bovine milk based on SPE with graphitized carbon black, followed by LC–MS/MS have been determined by Bogialli et al. [31]. However, up to date, no method has been published for a selective extraction, clean up and simultaneous determination of bensulfuron methyl, chlorsulfuron, metsulfuron methyl, primisulfuron methyl, rimsulfuron, thifensulfuron methyl, triasulfuron and tribenuron methyl residues in milk.

The purpose of the present work was to develop a rapid, selective, sensitive and reliable method for the simultaneous determination of the selected eight sulfonylurea herbicides from bovine milk samples using liquid chromatography in combination with diode array detection. To the best of our knowledge this is the first time that a single extraction – clean up step with Chem Elut cartridges has been applied to the simultaneous determination of sulfonylurea herbicides in milk samples. This effective extraction–clean up procedure allows to achieve clear extracts, no matrix effect and detection limits lower than the default limit of 0.01 mg kg^{-1} at the same time.

Validation of the proposed procedure [32–37] has been performed according to the European Commission Decision 657/2002/EC [38].

2. Experimental

2.1. Reagents, standards and samples

Certificated analytical standards of bensulfuron methyl (99.5% purity), chlorsulfuron (99.8%), metsulfuron methyl (99.3%), primisulfuron methyl (99.9%), rimsulfuron (99.2%), thifensulfuron methyl (96.4%), triasulfuron (97.3%) and tribenuron methyl (99.5%) were obtained from Riedel-De Haën (Seelze-Hannover, Germany). Common names and structures of the sulfonylurea herbicides evaluated here are shown in Fig. 1.

Acetonitrile and water (HPLC grade) were purchased from Carlo Erba (Milan, Italy). Pesticide quality solvents, dichloromethane and ethyl acetate, were supplied from Merck (Darmstadt, Germany). Chem Elut SPE cartridges packed with diatomaceous earth material were purchased from Varian (Harbor City, CA, USA).

Analytical standard stock solutions of each herbicide at 0.8 mg mL^{-1} were prepared in acetonitrile, by dissolving weighted exactly 40 mg of each analyte in 50 mL of acetonitrile and working standard solutions were obtained at various concentrations by dilution of the stock solutions in acetonitrile 0.01% solution of acetic acid (45:55, v/v). These solutions were stored under refrigerator conditions (4 ± 3 °C) and protected from light; under these conditions they are stable for at least 6 months. A standard multicomponent solution (10 μg mL^{-1}) was prepared by diluting each primary standard solution with the chromatographic mobile phase and was used for spiking milk samples, for preparing matrix-matched calibration standards in milk blank and for studying the linear dynamic range of the HPLC–DAD detection. The standard solutions were stored under refrigerator conditions (4 ± 3 °C) and protected from light; under these conditions the standard solutions are stable for at least 6 months.

Pasteurized, homogenized whole milk samples were purchased from local markets and tested not to contain the SUs included in the study at the method detection limits. Finally, each matrix was used for each experiment.

2.2. Extraction and cleanup procedure

Portions (5 g) of milk were transferred on top of a dry Chem Elut cartridge. After the liquid has drained into the cartridge wait for 15 min in order to obtain an even distribution on the filling material. A 32 mm × 0.70 mm I.D. Luer Lock needle was attached to the lower tip as a flow restrictor and the column was eluted with three 8-mL aliquots of dichloromethane/ethyl acetate (9:1, v/v).

The eluates were evaporated under vacuum to a small volume at room temperature and the last solvent traces were then removed by manually rotating the collecting flask. Residues were redissolved with 1 mL of mobile phase. Evaporation of the extracts and reconstitution in low volumes of mobile phase was necessary in order to reach an adequate preconcentration of pesticides that allowed to obtain low limits of detection (LOD).

2.3. HPLC–DAD system and operating conditions

The HPLC system consisted in a continuous vacuum degasser, a P4000 quaternary pump and a UV6000LP detector linked to a personal computer running the ChromQuest-version 4.2 software program (ThermoQuest, Milano, Italy).

Chromatographic separation of the target SUs was performed on a Synergi Hydro-RP-C18 (250 mm × 4.6 mm I.D., 4 μm particle size) column protected by RP18 guard column, both from Phenomenex (Torrance, CA, USA). A binary mobile phase with a gradient programme was used, combining solvent A (acetonitrile) and solvent B (0.01% solution of acetic acid) as follows: 45% A (9 min); 45–60% A (6 min); 60% A (5 min). The HPLC system was re-equilibrated with the initial composition for 3 min, prior to next injection. The flow rate was 1 mL min^{-1}. The injection volume was 20 μL. The external standard method of calibration was used for this analysis. The injection was performed three times to test the instrumental repeatability. Calibration curves were obtained by plotting peak areas against concentrations of analytes injected. Fig. 2 shows the respective UV spectra, absorption maxima and retention times of the target compounds.

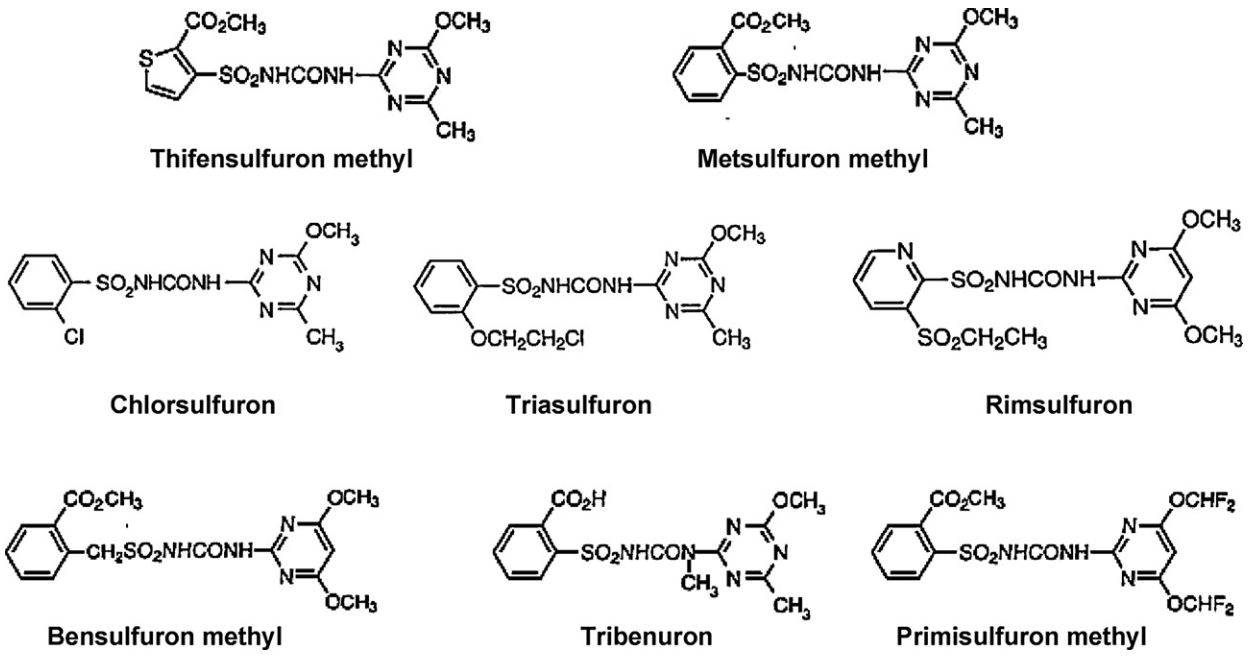

Fig. 1. Names and structures of eight sulfonylurea herbicides evaluated.

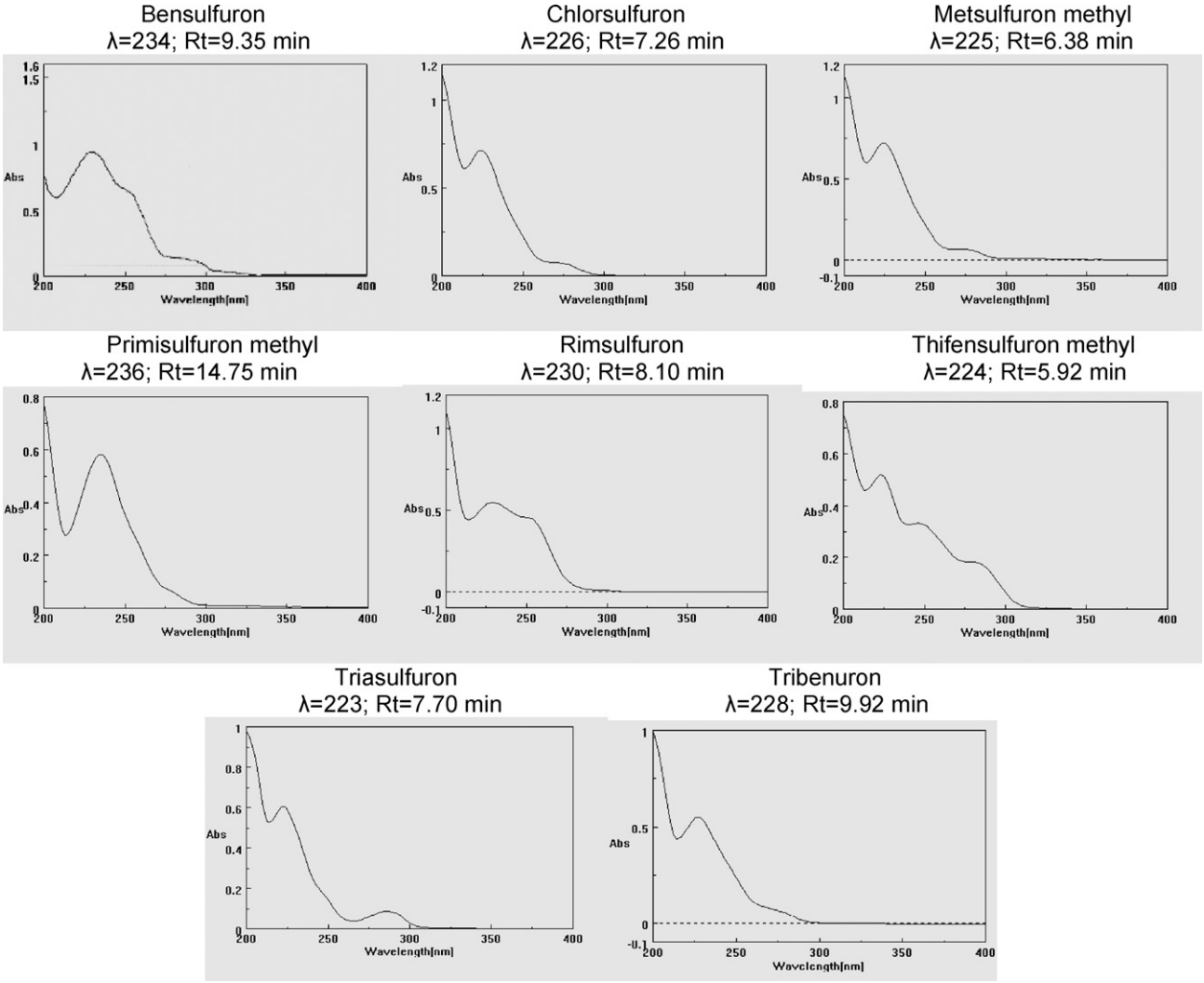

Fig. 2. UV spectra, absorption maxima and retention times of the examined SU's.

2.4. Validation study according to the 2002/657/EC decision

The analytical procedure was validated in terms of linear dynamic range, accuracy (recovery), precision (relative standard deviation, RSD) and selectivity.

Linearity was determined by calibration plots of matrix matched calibration standards, prepared by spiking blank milk extracts at five concentrations of standard working solution (0.025, 0.05, 0.075, 0.25 and 0.5 $\mu g\,mL^{-1}$), corresponding at five fortification levels (0.005, 0.010, 0.015 0.050 and 0.100 $mg\,kg^{-1}$).

To verify the absence of interfering substances around the retention time of analytes, 20 milk samples were analyzed.

The accuracy and precision were assessed by fortification of milk samples. Recovery and repeatability experiments were carried out, in six replicates, at three fortification levels of 0.5, 1 and 1.5 the MRL, corresponding to 0.005, 0.010 and 0.015 $mg\,kg^{-1}$, respectively, by adding known volumes of pesticide standards in the mobile phase to whole milk samples. Concentrations were calculated by comparing peak areas relatives to each analyte with those obtained from matrix-matched standards. At each level, the analyses were performed in six replicates to find the mean concentration and standard deviation of the fortified samples.

In order to check the stability of the analytes in matrix, a blank milk sample was divided into five aliquots and each aliquot was fortified at the LOQ level for each pesticide. One aliquot was analyzed immediately and the remaining aliquots were stored at $4 \pm 3\,^{\circ}C$ and analyzed after 1, 2, 4 and 20 weeks. The concentration of the analytes in each aliquot was shown to be stable within 20 weeks. Consequently, the extract could be stored for at least 3 months before instrumental determination.

3. Results and discussion

3.1. Development and optimization of the SPE–LC/UV–DAD method

The determination of pesticide residues in milk typically presents problems because many time consuming clean-up steps are often required to eliminate fat extracted along with the analyte of interest, that can interfere with the matrix. Solid-phase extraction has been successfully proposed for extracting pesticide residues from milk samples [19–21,25,26,28,31].

In the extraction step, whole bovine milk samples are added to the top of the dry Chem Elut cartridge. For the extraction method, different organic solvents were evaluated: acetonitrile, ethyl acetate, dichloromethane and in different mixtures and/or different ratios. The best results were obtained using a single extraction with the dichloromethane–ethyl acetate mixture (9:1, v/v): in these conditions no further clean up of the extracts was necessary, providing the best recoveries (78.4–99.7%) and reaching very low detection limits for the target analytes. This fast procedure requires no sample preparation or pretreatment as deproteinization and defattening, no drying step with nitrogen flow and provides, in a single step, adequate extraction-clean up of the analytes from a lipid matrix.

At first, separation was carried out with isocratic elution and the mobile phase consisted of mixture of acetonitrile/0.01% solution of acetic acid (45/55, v/v), obtaining good separation in a relatively short time (33 min). Afterwards, to reduce analysis time, a linear gradient elution has been used, starting from acetonitrile 45% and achieving a final concentration of 60% in about 20 min. The additional equilibration at the initial mobile phase composition resulted in a total analysis time of approximately 23 min, with respect to peak sharpness and chromatographic separation, Fig. 3. For these reasons this method was selected.

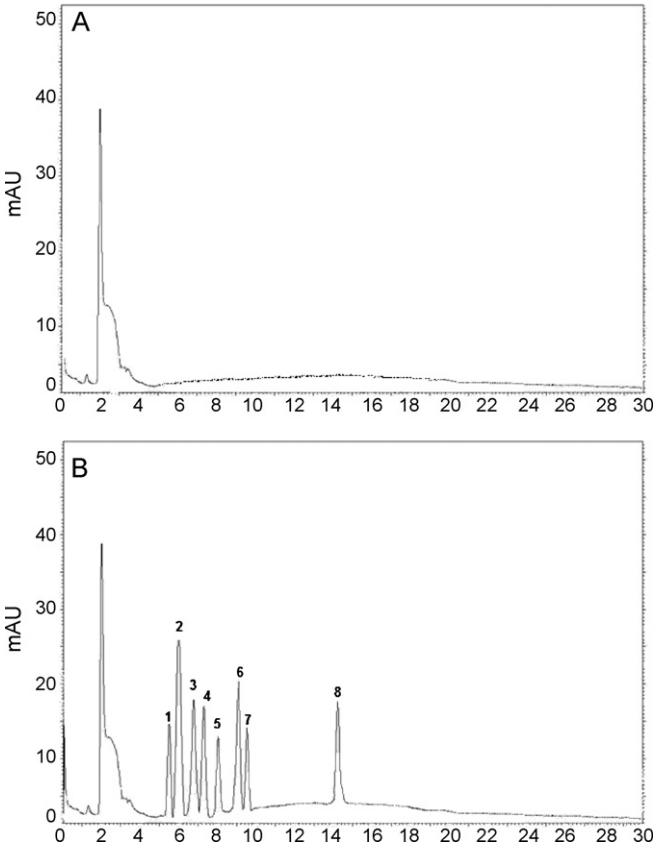

Fig. 3. HPLC–DAD chromatograms measured at λ 230 nm after extraction with Chem Elut cartridge of (A) a blank milk sample and (B) a spiked milk sample with 0.01 $mg\,kg^{-1}$ of each herbicide. Peaks: (1) Thifensulfuron methyl; (2) Metsulfuon methyl; (3) Chlorsulfuron; (4) Triasulfuron; (5) Rimsulfuron; (6) Bensulfuron methyl; (7) Tribenuron methyl; (8) Primisulfuron methyl.

In the case of UV–DAD, detection at 230 nm was the most sensitive and selective, with minimal absorbance by interfering compounds. During elution, spectra of single compounds can be acquired with the DAD. Generally, no blank milk extract, measured at the band maxima of UV spectra for each herbicide, shows any interfering peaks at the retention times of the examined analytes, Fig. 3. In Fig. 2 the UV spectra, the absorption maxima and the retention times of the target compounds are shown.

3.2. Method validation

As EU requires, validation of analytical methods for the determination of residues of pesticides in food was made according to the criteria specified in the Commission Decision 2002/657/EC [38]. The validation procedure included the determination of the linearity, specificity, accuracy, precision and sensitivity of the method by calculation of both decision limit (CC_α) and detection capability (CC_β).

Detector linearity was determined by calibration plots constructed through the range from 0.01 to 1.0 $\mu g\,mL^{-1}$ for the 8 sulfonylurea herbicides at 5 concentration levels. The UV–DAD detector gave linear response over the studied range of concentrations and the least-squares linear regression analysis of the data provided excellent coefficient of determination (r^2) values for all compounds tested ($r^2 > 0.999$) and residuals not exceeding ±9.8%, thus indicating a good fit of the calibration function. The calibration was performed by the use of matrix-matched calibration standards prepared as described in Section 2.4. The quantitation of the samples was performed using the means of two 3-(4) point calibration

Table 1
Linear regression data for matrix matched calibration standards and solvent (chromatographic mobile phase) calibration standards.

Herbicide	Slope[a]	r^2	Residuals (%)	Matrix effect[b]
Bensulfuron methyl	Solvent 903.16	0.9988	±0.1	1.010
	Matrix 912.09	0.9983	±1.9	
Chlorsulfuron	Solvent 547.41	0.9991	±5.5	1.028
	Matrix 563.04	0.9990	±9.8	
Metsulfuron methyl	Solvent 673.58	0.9993	±1.4	1.016
	Matrix 684.55	0.9992	±3.9	
Primisulfuron methyl	Solvent 976.45	0.9994	±4.6	1.015
	Matrix 991.68	0.9990	±9.5	
Rimsulfuron	Solvent 627.99	0.9998	±7.2	1.001
	Matrix 628.47	0.9985	±9.8	
Thifensulfuron methyl	Solvent 956.39	0.9994	±6.3	1.011
	Matrix 967.35	0.9991	±8.7	
Triasulfuron	Solvent 457.04	0.9987	±2.0	1.058
	Matrix 483.80	0.9985	±4.1	
Tribenuron	Solvent 775.45	0.9998	±0.2	1.011
	Matrix 783.76	0.9997	±1.5	

[a] (×103).
[b] Slope matrix/slope solvent.

curves. The matrix effect of the present method was investigated by comparing standards in solvent (chromatographic mobile phase) with matrix-matched standards. Table 1 summarizes the analytical results obtained for each pesticide in solvent and in matrix, showing that the matrix effect is negligible in determining the target compounds.

To verify specificity, a representative number of blank milk extracts ($n = 20$) were analyzed in order to check the absence of potential interfering peaks at the retention times of the target Sus. Generally, no blank milk extract, measured at the band maxima of UV spectra for each herbicide, shows any interfering peaks at the retention times of the examined analytes. Chromatograms of spiked milk samples were quite similar to those obtained with the standard solution of pure herbicides. As can be seen in Fig. 3, the HPLC–DAD chromatogram of unspiked milk extract shows good baseline stability with no interfering peaks at the retention times of the considered compounds, indicating that the proposed clean-up procedure is effective and suitable for the determination of the target analytes.

To evaluate the accuracy of the present method, standard mixture solution of the eight selected sulfonylurea herbicides was added to milk samples, at fortification levels of 0.5, 1 and 1.5 the MRL. The mean recoveries of the pesticides ($n = 6$) for each fortification level are listed in Table 2. In all instances, satisfactory results were found, with recovery values between 78.4% and 99.7%, not influenced by the spiking level. These values meet the requirements of the European Commission [36,37] for validation recoveries indicating that a method can be considered accurate and precise when the accuracy of data is between 70 and 110%, with relative standard deviations (RSDs) not higher than 20%.

3.2.1. Precision

The precision of the method was determined by between-day and within-day studies, expressed by the relative standard deviation (RSD) and calculated using the double measurement of peak area of each herbicide in the matrix. The within-day precision RSDr was measured by comparing standard deviation of the recovery percentages spiked milk samples run the same day. The between-day precision RSD_R was determined by analyzing spiked milk samples for four alternate days. Replicated ($n = 6$ for each concentration level) samples were all run and the RSD value was calculated for each herbicide. The method was found to be precise (RSD < 10%) for all the compounds studied at all spiking levels (Table 2).

The limit of detection (LOD) and the limit of quantification (LOQ) (corresponding to the analyte amount for which the area is equal to 3 times and 10 times the chosen standard deviation, respectively) were calculated from ordinary least squares regression data [34]. To calculate LOD and LOQ values the chosen standard deviation was the intercept standard deviation.

The standard deviation chosen to calculate the LOD and LOQ values is the residual standard deviation of the regression line for all sulfonylurea herbicides in the analyzed matrix, Table 3.

The quality and the correct interpretation of the analytical results attained by control official laboratories are ensured by the Commission Decision 2002/657/EC, by introducing two new parameters, decision limit CC_α and detection capability CC_β, that replace the old concepts of limit of detection and limit of quantitation. CC_α is the limit at and above which it can be concluded with an error probability of 5% that a sample is not compliant, whereas CC_β means the smallest content of the substance that may be detected, identified and/or quantified in a sample with an error probability of β. Being the LOQs equal to or lower than the default maximum

Table 2
Recovery (%), within-day precision (RSDr, %) and between-day precision (RSDR, %) of the herbicides from spiked blank milk samples($n = 6$).

Herbicide	Fortification level 0.005 mg kg^{-1} (0.5 MRL)			Fortification level 0.010 mg kg^{-1} (1.0 MRL)			Fortification level 0.015 mg kg^{-1} (1.5 MRL)		
	Mean (%)	RSD_r (%)	RSD_R (%)	Mean (%)	RSD_r (%)	RSD_R (%)	Mean (%)	RSD_r (%)	RSD_R (%)
Bensulfuron methyl	87.2	2.4	5.1	87.0	1.9	7.8	86.8	1.6	4.2
Chlorsulfuron	90.3	5.6	6.7	89.1	6.7	4.9	93.8	9.8	3.3
Metsulfuron methyl	93.3	3.3	3.5	92.9	4.5	3.1	90.6	2.8	4.6
Primisulfuron methyl	95.7	3.9	6.9	93.4	4.6	8.5	99.7	3.6	9.9
Rimsulfuron	79.5	4.0	3.3	78.8	1.9	2.1	79.8	2.1	4.5
Thifensulfuron methyl	91.6	1.2	5.1	88.5	3.2	3.4	93.8	0.8	3.4
Triasulfuron	94.8	2.3	3.0	95.9	1.4	2.9	95.6	2.4	2.4
Tribenuron	78.8	2.0	2.7	78.4	1.2	1.8	80.0	0.3	3.2

Table 3
LODs and LOQs values obtained from ordinary least-squares regression data and calculations of error α and β as well as decision limits (CC_α) and (CC_β) at the LOQ levels of the method.

Herbicide	LODmg kg^{-1}	LOQ (Fortification level) mg kg^{-1}	Measured ± SD mg kg^{-1}	RSD	Recovery (%)	Error α (1.64 × SD)	CC_α mg kg^{-1}
Bensulfuron methyl	0.002	0.008	0.0070 ± 0.0001	1.9	87.0	0.0002	0.0082
Chlorsulfuron	0.003	0.009	0.0080 ± 0.0005	6.7	89.1	0.0008	0.0098
Metsulfuron methyl	0.002	0.007	0.0065 ± 0.0003	4.5	92.9	0.0005	0.0075
Primisulfuron methyl	0.002	0.009	0.0084 ± 0.0004	4.6	93.4	0.0006	0.0096
Rimsulfuron	0.003	0.01	0.0079 ± 0.0002	1.9	78.8	0.0003	0.0103
Thifensulfuron methyl	0.003	0.01	0.0088 ± 0.0003	3.2	88.5	0.0002	0.0102
Triasulfuron	0.003	0.009	0.0086 ± 0.0001	1.4	95.9	0.0002	0.0092
Tribenuron	0.004	0.01	0.0078 ± 0.0001	1.2	78.4	0.0002	0.0102

Herbicide	CC_α (Fortification level) mg kg^{-1}	Measured ± SD mg kg^{-1}	RSD	Recovery (%)	Error β (1.64 × SD)	CC_β mg kg^{-1}
Bensulfuron methyl	0.0082	0.0071 ± 0.0002	2.3	87.0	0.0003	0.0085
Chlorsulfuron	0.0098	0.0078 ± 0.0002	3.1	89.1	0.0004	0.0102
Metsulfuron methyl	0.0075	0.0069 ± 0.0001	1.7	92.9	0.0002	0.0077
Primisulfuron methyl	0.0096	0.0090 ± 0.0004	4.2	93.4	0.0006	0.0102
Rimsulfuron	0.0103	0.0081 ± 0.0003	3.9	78.8	0.0005	0.0108
Thifensulfuron methyl	0.0102	0.0090 ± 0.0004	4.7	88.5	0.0007	0.0109
Triasulfuron	0.0092	0.0088 ± 0.0004	4.4	95.9	0.0006	0.0098
Tribenuron	0.0102	0.0080 ± 0.0002	2.5	78.4	0.0003	0.0103

residue limits established by European legislation at 0.01 mg kg^{-1}, the CC_α values were calculated by spiking 20 blank milk samples at the LOQ levels of the method for each herbicide. The spiking level concentration, plus 1.64 times the corresponding standard deviation, represents the CC_α. Then, the CC_β values were calculated by analyzing 20 blank spiked samples at corresponding calculated CC_α level for each analyte. The concentration at the CC_α, plus 1.64 times the corresponding standard deviation, equals the CC_β. In Table 3 are listed the obtained CC_α and CC_β for the target compounds.

4. Conclusions

This paper describes for the first time a fast, simple and sensitive analytical method based on SPE–HPLC–DAD was developed and validated for the simultaneous determination of eight sulfonylurea herbicide residues in whole bovine milk.

The single extraction procedure of the described method is very simple and requires no sample preparation or pre-treatment, providing adequate clean-up of the lipid matrix. Whole milk extracts are very clean, with no interfering peaks at the retention time of the target compounds, indicating good selectivity of the proposed method.

Moreover, gradient elution by the mobile phase acetonitrile-water yields good separation and resolution and the analysis time required for the chromatographic determination of the eight sulfonylurea herbicides is very short (around 20 min for a chromatographic run).

Satisfactory validation parameters such as linearity, recovery, precision and very low limits were obtained and, according to the guidelines of European Commission 2002/657/EC, decision limit (CC_α) and detection capability (CC_β) have been calculated.

For all of the herbicides the sensitivity of the method was good enough to ensure reliable determination al levels lower than the respective MRLs.

Therefore, the proposed analytical procedure could satisfactorily be useful for regular monitoring of sulfonylurea herbicides residues on a large number of bovine whole milk samples.

References

[1] W.R. Leeman, K.J. Van Den Berg, G.F. Houben, Food Addit. Contam. 24 (2007) 1.
[2] C.A. Kan, G.A.L. Meijer, Anim. Feed Sci. Technol. 133 (2007) 84.
[3] T. Cserháti, E. Forgács, Z. Deyl, I. Miksik, A. Eckhardt, Biomed. Chromatogr. 18 (2004) 350.
[4] C. Soler, K.J. James, Y. Picó, J. Chromatogr. A 1157 (2007) 73.
[5] Council Directive EEC/363/86 of July 24, 1986 on the fixing of maximum levels for pesticide residues in and on foodstuff of animal origin of European Union, Brussels, 1986.
[6] Regulation EC/396/2005 of the European Parliament and of the Council of February 23, on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC, Strasbourg, 2005.
[7] Joint FAO/WHO food standards programme Codex Committee on pesticide residues, Fortieth Session, Hangzhou, China, April 14–19, 2008, Milk and milk fat maximum residue limits.
[8] Commission Regulation EC/149/2008 of January 29amending Regulation (EC) No 396/2005 of the European Parliament and of the Council by establishing Annexes II, III and IV setting maximum residue levels for products covered by Annex I thereto, Brussels, 2008.
[9] Commission Directive EC/11/2009 of February 18 amending Council Directive 91/414/EEC to include bensulfuron, sodium 5-nitroguaiacolate, sodium o-nitrophenolate, sodium p-nitro phenolate and tebufenpyrad as active substances, Brussels, 2009.
[10] http://ec.europa.eu/sanco_pesticides/public/index.cfm.
[11] A.K. Sarmah, J. Sabadie, J. Agric. Food Chem. 50 (2002) 6253.
[12] S. Sondhia, Bull. Environ. Contam. Toxicol. 83 (2009) 799.
[13] S. Ishimitsu, A. Kaihara, K. Yoshii, Y. Tsumura, Y. Nakamura, Y. Tonogai, J. Health Sci. 48 (2002) 335.
[14] A.J. Krynitsky, J. AOAC Int. 80 (1997) 392.
[15] P. Degelmann, S. Egger, H. Jürling, J. Müller, R. Niessner, D. Knopp, J. Agric. Food Chem. 54 (2006) 2003.
[16] S. Polati, M. Bottaro, P. Frascarolo, F. Gosetti, V. Gianotti, M.C. Gennaro, Anal. Chim. Acta 579 (2006) 146.
[17] F. Perreau, P. Bados, L. Kerhoas, S. Nélieu, J. Einhorn, Anal. Bioanal. Chem. 388 (2007) 1265.
[18] G. Stoev, A. Stoyanov, J. Chromatogr. A 1150 (2007) 302.
[19] A. Laganà, G. Fago, A. Marino, B. Pardo-Martinez, Chromatographia 38 (1994) 88.
[20] A. Laganà, A. Marino, G. Fago., Chromatographia 41 (1995) 178.
[21] L. Balduini, M. Matoga, E. Cavalli, E. Seilles, D. Riethmuller, M. Thomassin, Y.C. Guillaume, J. Chromatogr. B 794 (2003) 389.
[22] C. Basheer, H.K. Lee., J. Chromatogr. A 1047 (2004) 189.
[23] L.Y. Zhu, K.H. Ee, L.M. Zhao, H.K. Lee, J. Chromatogr. A 963 (2002) 335.
[24] M. Brutti, C. Blasco, Y. Pico., J. Sep Sci. 33 (2010) 1.
[25] G. Pagliuca, T. Gazzotti, E. Zironi, P. Sticca, J. Chromatogr. A 1071 (2005).
[26] F. Bordet, D. Inthavong, J.M. Fremy, J. AOAC Int. 85 (2002) 1398.
[27] M. Mezcua, M.R. Repetti, A. Aguera, C. Ferrer, J.F. Garcia-Reyes, A.R. Fernandez-Alba., Anal. Bioanal. Chem. 389 (2007) 1833.
[28] S. Seccia, P. Fidente, D. Montesano, P. Morrica, J. Chromatogr. A 1214 (2008) 115.
[29] C.H.P. Ciscato, A.B. Gebara, H.D. Spinosa, J. Environ. Sci. Health Part B 37 (2002) 323.
[30] T. Dagnac, M. Garcia-Chao, P. Pulleiro, C. Garcia-Jares, M. Llompart, J. Chromatogr. A 1216 (2009) 3702.

[31] S. Bogialli, R. Curini, A. Di Corcia, A. Laganà, A. Stabile, E. Sturchio, J. Chromatogr. A 1102 (2006) 1.
[32] R. Causon, J. Chromatogr. B 689 (1997) 175.
[33] A.R.C. Hill, S.L. Reynolds, Analyst 124 (1999) 953.
[34] J. Vial, A. Jardy, Anal. Chem. 71 (1999) 2672.
[35] G.M.F. Pinto, I.C. Jardim, J. Chromatogr. A 869 (2000) 463.
[36] Document SANCO/825/00 rev.7 – Guidance document on residue analytical methods. European Commission, Directorate General Health and Consumer Protection, Brussels, March 17, 2004.
[37] Document SANCO/3131/2007 on method validation and quality control procedures for pesticide residues analysis in food and feed, October 31, 2007, http://www.crl-pesticides.eu/library/dox/srm/AqcGuidance.pdf, Revised version SANCO/10684/2009.
[38] Commission Decision EC/657/2002 of August 12, implementing Council Directive EC/23/96 on the performance of analytical methods and the interpretation of results, European Union, Brussels, 2002.