Analytical Method for the Determination of Atrazine and Its Dealkylated Chlorotriazine Metabolites in Urine by Gas Chromatography/Mass Selective Detection

Liakat H. Bodalbhai, Robert A. Yokley,* and Max W. Cheung

Development Resources/Chemical Support Department, Novartis Crop Protection, Inc., Greensboro, North Carolina 27419

A multiresidue method is reported for the determination of atrazine and its chloro dealkylated metabolites in urine. Urine samples were subjected to a protein precipitation procedure followed by further purification using strong anion exchange and silica solid-phase extraction columns. Final analysis was accomplished using gas chromatography/mass selective detection in the selected ion monitoring mode. The limits of detection were 0.050 ng injected for 2,4-diamino-6-chloro-*s*-triazine (G-28273) and 0.025 ng injected for 2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine (atrazine), 2-amino-4-chloro-6-(isopropylamino)-*s*-triazine (G-30033), and 2-amino-4-chloro-6-(ethylamino)-*s*-triazine (G-28279). The limits of quantification were 1.0 ppb for atrazine and G-30033 and 2.0 ppb for G-28279 and G-28273. The mean procedural recoveries obtained during the method validation were 115, 113, 112, and 97% and the standard deviations were 10.6, 10.2, 9.5, and 16.1% for atrazine, G-30033, G-28279, and G-28273, respectively. Additional recovery data were obtained during a worker exposure study and during an outside ruggedness trial. All studies were conducted under U.S. EPA FIFRA Good Laboratory Practice Standards 40 CFR 160.

Keywords: *Urine; atrazine; chlorodealkylated triazine metabolites; human exposure; gas chromatography/mass selective detection (GC/MSD); selected ion monitoring (SIM); Good Laboratory Practices (GLP)*

INTRODUCTION

Atrazine is a restricted-use herbicide manufactured by several agricultural chemical companies and sold under various trademarks (e.g., AAtrex). It is most often used in corn, sorghum, and sugarcane production for the control of annual broadleaf and grass weeds and may be applied pre- or post-emergence. It metabolizes in plants and animals and undergoes environmental degradation to form dealkylated chlorotriazine metabolites (Esser et al., 1975; Shimabukuro et al., 1971). Their structures and chemical names are shown in Figure 1.

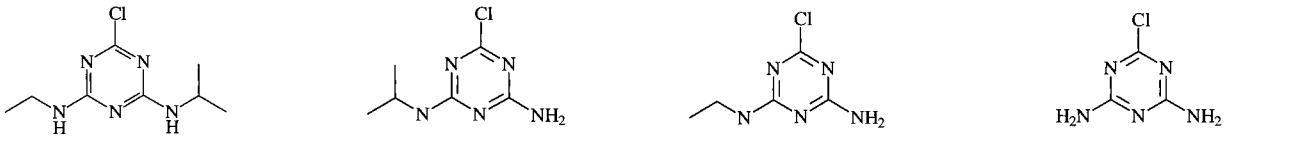
Applicators, mixers, and loaders who mix, spray, or apply atrazine to corn and other crops face potential dermal and/or inhalation exposure when handling bulk quantities of the formulated active ingredient. To estimate this potential exposure, Novartis Crop Protection, Inc. (formerly Ciba Crop Protection), conducted two extensive worker exposure studies in 1995 that resulted in the collection of >1600 urine samples. The studies were designed to provide information necessary for an assessment of the internal exposure, nature of the residue in man, excretion kinetics, and utility of chlorotriazine data for biomonitoring "human exposure". The results of these studies are reported elsewhere (Selman et al., 1996; Atrazine/Simazine, 1996).

Existing urine methodology for these compounds is restricted to limits of quantification (LOQ) that are higher than desirable for the purposes of these worker exposure studies and/or require the use of two separate methods to analyze all four compounds. LOQ reported by other workers using gas chromatography with mass selective detection (GC/MSD) for the final determination include 10 ppb for atrazine, G-30033, and G-28279 and 1 ppm for G-28273 (Lucas et al., 1993). Others using conventional detection systems reported a LOQ of 20 ppb for atrazine using GC/flame ionization detection (FID) or nitrogen−phosphorus detection (NPD) but with no provision for the analysis of the dealkylated chlorotriazine metabolites (Kumazawa et al., 1992). Bradway and Moseman (1982) reported 100 ppb for each analyte using GC/NPD. One group using GC/NPD reported average recoveries of 95% for urine samples fortified in the 0.10−100 ppb range for all four analytes, but representative chromatography and detailed recovery information at the low fortification levels were not presented (Catenacci et al., 1993). Method validation following Good Laboratory Practices (GLP) standards was not mentioned in any of these papers.

Thus, a GLP-validated analytical method acceptable to the U.S. EPA was needed to support the worker exposure studies in which all four analytes could be measured accurately, precisely, and reliably at the desired screening level using one procedure. Urine samples were fortified with the four analytes from 1.0 to 200 ppb and subjected to the sample preparation procedures outlined in Novartis Analytical Method AG-637. Final analyses were accomplished utilizing GC/MSD in the selected ion monitoring (SIM) mode.

* Author to whom correspondence should be addressed [e-mail (robert.yokley@cp.novartis.com; fax (910) 632-7645].

S0021-8561(97)00397-X CCC: $15.00
Published on Web 01/19/1998



Atrazine
2-Chloro-4-ethylamino-6-isopropylamino-s-triazine

6-chloro-.N.-ethyl-.N.-isopropyl-[1,3,5]triazine-2,4-diamine (IUPAC)

CAS #1912-24-9

G-30033
2-Amino-4-chloro-6-isopropylamino-s-triazine

6-chloro-.N.-isopropyl-[1,3,5]triazine-2,4-diamine (IUPAC)

CAS #6190-65-4

G-28279
2-Amino-4-chloro-6-ethylamino-s-triazine

6-chloro-.N.-ethyl-[1,3,5]triazine-2,4-diamine (IUPAC)

CAS # 1007-28-9

G-28273
2,4-diamino-6-chloro-s-triazine

6-chloro-[1,3,5]triazine-2,4-diamine (IUPAC)

CAS #3397-62-4

Figure 1. Structures and chemical names of atrazine and its chloro dealkylated metabolites.

EXPERIMENTAL PROCEDURES

Solvents and Reagents. HPLC grade solvents acetonitrile (A998-4), methanol (A452-2), ethyl acetate (E195-4), Optima grade acetone (A929-4), and ACS grade sodium chloride (S271-3), sodium bicarbonate (S233-3), sodium hydroxide solution (SS254-500), sodium carbonate anhydrous (S263-500), sodium sulfate (S421-3), Celite 545 (C212-500), and sulfuric acid (A300-212) were all obtained from Fisher Scientific Co.

Preparation of Solutions. The pH 10 buffer solution was prepared by mixing 500 mL of 0.050 M $NaHCO_3$ with 107 mL of 0.10 M NaOH in a 1-L volumetric flask and diluting to the mark with deionized (DI) water (Dean, 1979).

Solid-Phase Extraction (SPE) Columns. A silica SPE column, 5 g (Waters, WAT036930), and a Megabond Elut quaternary ammonium strong anion exchange (SAX) column, 10 g (Varian, A1-122560-37), were used.

Standards. Analytical standards of atrazine (98.7%), G-30033 (99%), G-28279 (98%), and G-28273 (97%) were obtained from the Analytical and Product Chemistry Department, Novartis Crop Protection, Greensboro, NC. Separate stock solutions were prepared of 100 μg/mL atrazine and 50 μg/mL G-30033, G-28279, and G-28273 (corrected for percent purity). A 5.0 μg/mL mixed standard was prepared by combining aliquot portions of the four stock standards. Serial dilutions were made of the 5.0 μg/mL mixed standard to produce calibration standards in the range of 0.0125−1.0 μg/mL.

Mixed fortification standards at the 25 ng/mL concentration level for atrazine and G-30033 and the 50 ng/mL concentration level for G-28279 and G-28273 were prepared from the individual stock standards. Thus, 1.0 mL of this fortification standard added to 25 mL of urine (specific gravity of urine is typically 1.015−1.025 g/mL) provided the desired fortification levels at the method LOQ of 1.0 ppb for atrazine and G-30033 and 2.0 ppb for G-28279 and G-28273. Appropriate dilution of the mixed stock standard solution can be performed to obtain higher concentration fortification standards if desired. A maximum fortification standard volume of 2.0 mL or less is recommended.

Sample Storage. Urine samples to be analyzed for residues of atrazine and its chlorotriazine metabolites should be stored frozen until analyzed. The control urine samples used during this method validation were pooled donor samples from various members of the Product Safety Group, Novartis Crop Protection, Inc. The results of a storage stability study indicate that these compounds are stable in urine under freezer (−20 °C) storage conditions for at least 6 months and under refrigerator (4 °C) storage conditions for at least 8 weeks (Yokley et al., 1996). This study is still in progress to ascertain the maximum storage interval under freezer conditions.

Sample Preparation. A 25-mL aliquot portion of a well-mixed, homogeneous urine sample was transferred to an Erlenmeyer flask. Fortifications on control urine samples were performed at this time. Acetonitrile (20 mL) was added, the flask was vigorously shaken for 30 s, and the mixture was allowed to stand for 20 min. Celite 545 (0.50 g) was added, and the mixture was shaken for 10 s and then vacuum filtered through a Büchner funnel containing a Whatman No. 5 filter paper. The flask and filter pad were rinsed with 20 mL of deionized water. The filtrate was transferred to a round-bottom flask for concentration via rotary evaporation (water bath temperature of 35−40 °C). Acetonitrile (10 mL) was used to rinse the collection flask. The acetonitrile was removed from the filtrate (when water condensation appeared on the condenser coils) followed by the addition of 2 mL of 2 M H_2SO_4 and 5 mL of methanol. The pH was measured to ensure it was ≤3.0.

Table 1. Retention Time, Target and Qualifier Ions, and Qualifier/Target Ion Ratios Used for the GC/MSD Analyses

analyte	retention time[a] (min)	target ion (*m/z*)	qualifier ion (*m/z*)	Q1/Tgt0 ion ratio[b]	Q1/Tgt ion ± 20% acceptance range
G-28273	10.38	145	147	31.3	25.1−37.7
G-28279	11.23	158	160	32.3	25.3−37.9
G-30033	11.31	172	174	31.1	25.0−37.6
Atrazine	12.27	200	215	51.4	41.1−61.7

[a] This will vary according to column length and other operating parameters. [b] This confirmation ratio will vary slightly from analytical set to analytical set.

Table 2. Gas Chromatographic Parameters

parameter	value	parameter	value
oven, initial temp (°C)	80	ramp 2 rate (°C/min)	20
oven, initial time (min)	1	final temperature (°C)	275
injector temp (°C)	225	final time (min)	11
ramp 1 rate (°C/min)	15	column head pressure (0 time) (psi)	8
final temp (°C)	200		
final time (min)	3		

A 10-g Megabond Elut SAX column was conditioned with 15 mL each of methanol and 0.10 M H_2SO_4. The acidic aqueous filtrate was transferred to the SAX column and allowed to flow at ~2 drops/s, and the eluate was collected in a beaker. The round-bottom flask was rinsed with 20 mL of an acidic methanol solution (prepared by mixing 15 mL of 0.1 M H_2SO_4 with 5 mL of methanol), which was transferred to the SAX column. This eluate was collected in the same beaker. A gentle stream of N_2 was applied to the top of the SAX column for ~2 min to ensure complete removal of the eluate since the analytes are not retained on this column under these conditions.

The eluate from the SAX column was transferred to a 500-mL separatory funnel containing 10 g of NaCl and 2 g of Na_2CO_3. The funnel was vigorously shaken and intermittently vented to release the CO_2 generated during the neutralization of the acid. The eluate was partitioned with 40 mL of ethyl acetate after 5 mL of pH 10 buffer was added. In a few cases, emulsions formed, which were broken by the addition of 5 mL of methanol. The layers were separated, and 3 drops of 20% NaOH was added to the aqueous fraction. The basic aqueous fraction was further partitioned twice with 40-mL portions of ethyl acetate. The pooled ethyl acetate fractions were dried using anhydrous Na_2SO_4 and reduced to near dryness via rotary evaporation. The residues were reconstituted in 5 mL of ethyl acetate.

A 5-g silica SPE column was conditioned with 5 mL of methanol, 10 mL of acetonitrile, and 30 mL of ethyl acetate.

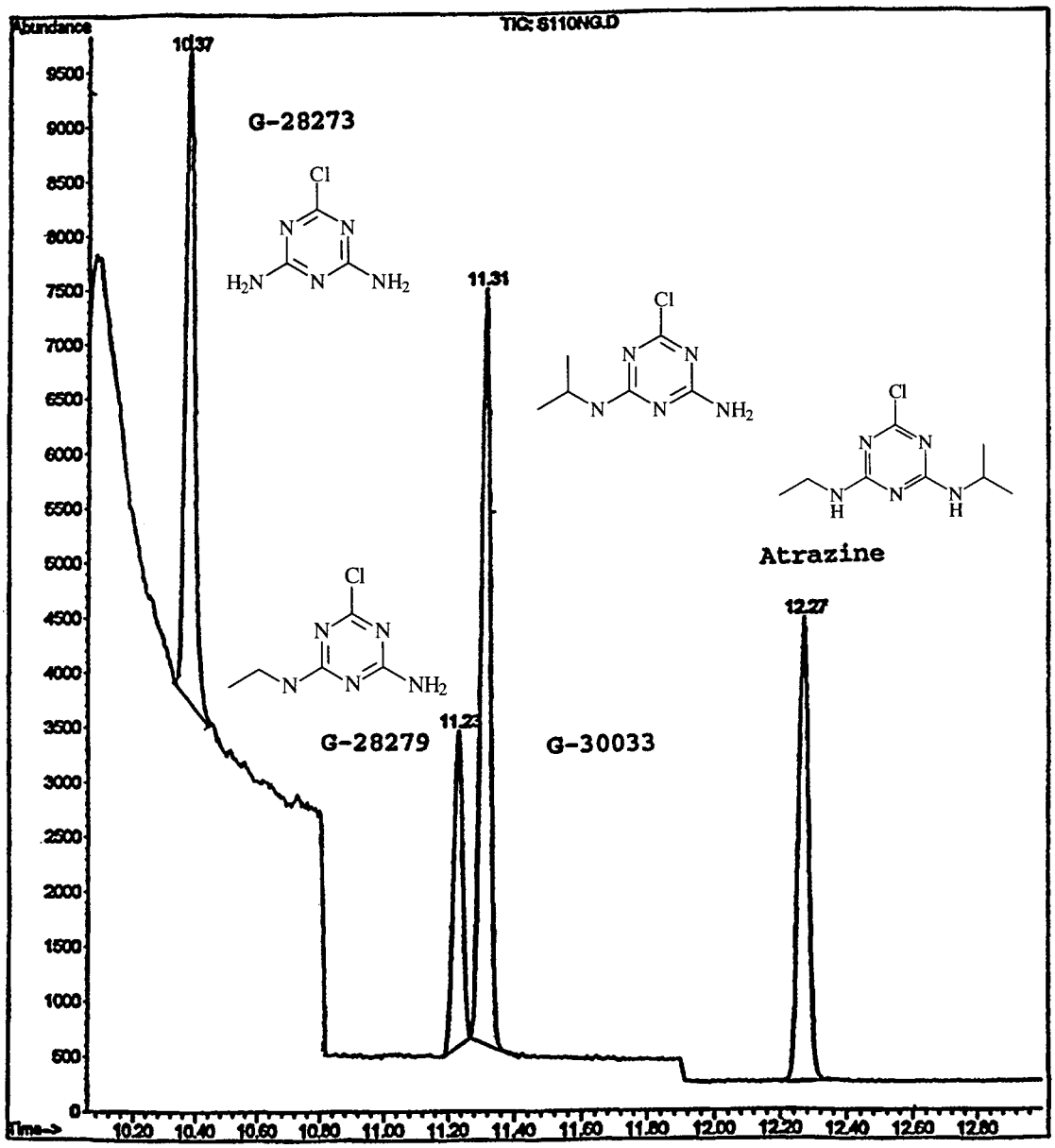


Figure 2. TIC of a mixed standard containing atrazine, G-30033, G-28279, and G-28273, each at the 0.10 ng injected concentration level.

The ethyl acetate fraction from the partitioning step was loaded and allowed to elute under gravity. The column was washed with 5 mL of ethyl acetate followed by elution of the analytes with 28 mL of ethyl acetate. The eluate was reduced to dryness via rotary evaporation and reconstituted in an appropriate volume of acetone (1.5 mL to achieve the method LOQ) for analysis using GC/MSD.

Instrumentation. Analyses were performed using either a Hewlett-Packard Model 5890 Series II gas chromatograph interfaced (capillary direct) to a 5972 mass selective detector (GC/MSD) or a Hewlett-Packard 6890 Series GC/MSD, both operated in the SIM mode. The ions of interest for each analyte, shown in Table 1, were obtained via electron ionization (EI) at 70 eV. The MSD transfer lines were maintained at 280 °C, and tuning was performed on a daily basis with perfluorotributylamine (PFTBA) to ensure accurate mass calibration. The GCs were equipped with splitless injectors and Supelco SPB-1301 (J&W DB-1301 can also be used), 0.25 mm i.d. × 30 m, 0.25 μm film thickness, capillary columns. Electronic pressure programming (EPP) was utilized in conjunction with the temperature programs detailed in Table 2.

System Suitability Testing. It is recommended that an analytical set consists of six analytical standards of various concentrations, a blank (acetone), control, one or two controls fortified with the four analytes for procedural recovery purposes, and 6−10 samples for analysis. A reagent blank should be initially included in each analytical set, but routine inclusion is not necessary as long as interference problems are not encountered. Additional standards should be dispersed throughout the run as a means of checking the stability of the system for variances in MSD sensitivity and/or column performance. Each analytical set should contain a minimum of two and should terminate with one of these "stability check" standards (Jenke, 1996).

RESULTS AND DISCUSSION

GC/MSD Analyses. A total ion chromatogram (TIC) of a mixed standard containing G-28273, G-28279, G-30033, and atrazine at the 0.10 ng injected level is shown in Figure 2. The sharp baseline changes result when switching ions for monitoring purposes (see Table 1). For example, the ions 145 and 147 are initially monitored, but after elution of G-28273, the ions for the next two analytes, 158 and 160 for G-28279 and 172

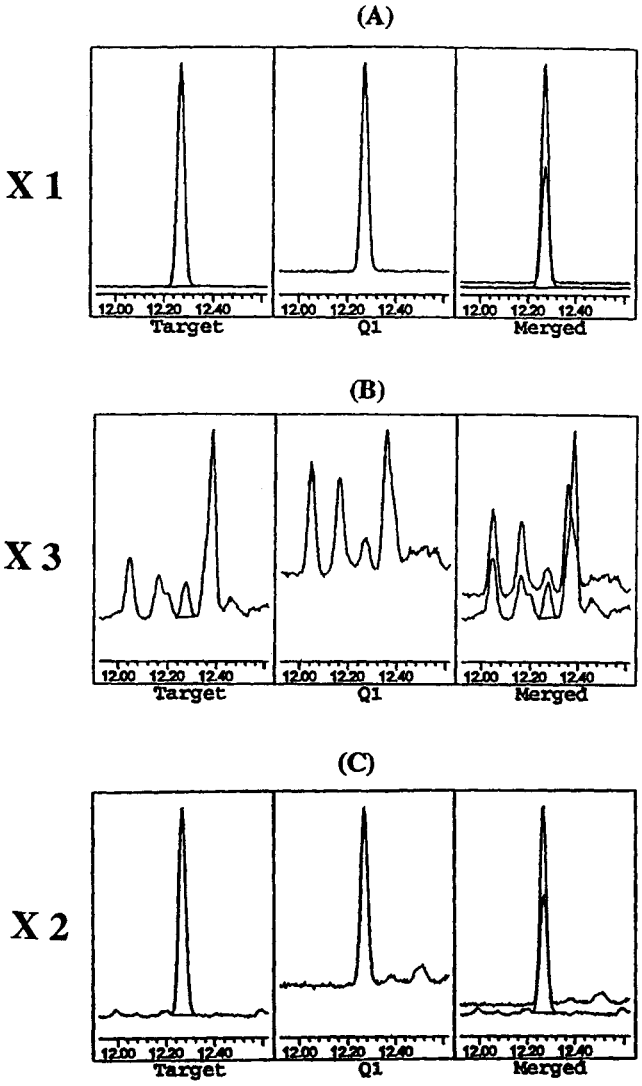


Figure 3. Representative SIM chromatograms for atrazine: (A) 0.10 ng injected standard, (B) control, and (C) 1.0 ppb procedural recovery.

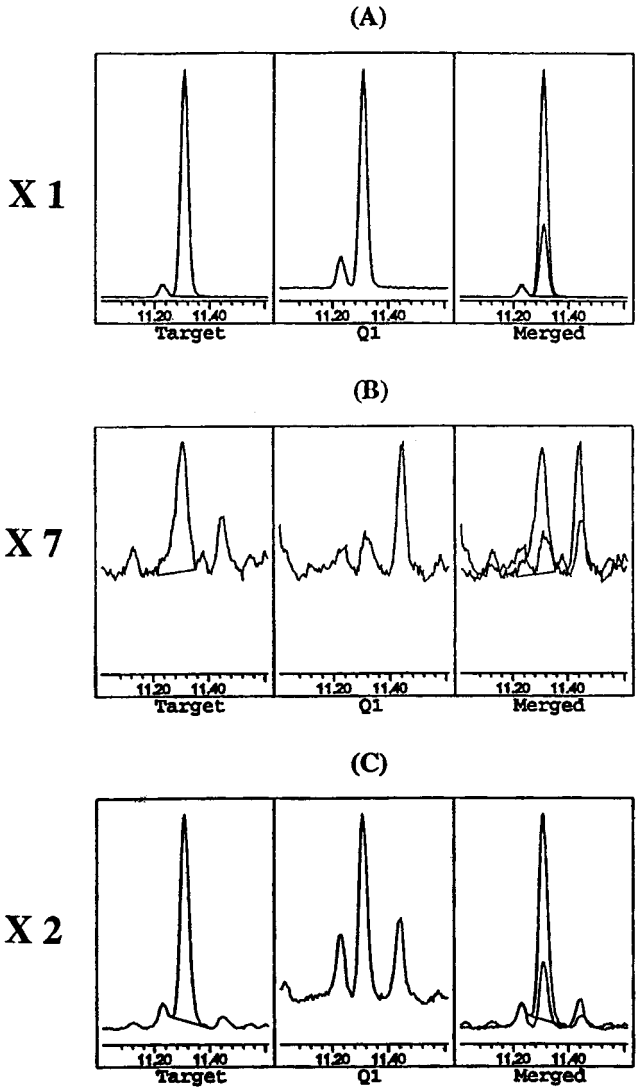


Figure 4. Representative SIM chromatograms for G-30033: (A) 0.10 ng injected standard, (B) control, and (C) 1.0 ppb procedural recovery.

and 174 for G-30033, are simultaneously monitored because of the small difference in their retention times. After elution of these two analytes, the ions 200 and 215 are monitored for atrazine. The TIC abundances (*y*-axis) depicted in Figure 2 are functions of the identity and the summation of the total number of each ion monitored and are shown only to illustrate the separation. It is the target (Tgt) and qualifier (Q1) ions, shown in Table 1, that are of analytical utility for quantification and confirmation, respectively.

Representative selected ion monitoring (SIM) chromatograms of a 0.10 ng injected standard, control, and 1.0 ppb procedural recovery sample for atrazine are shown in Figure 3. Note that inspection of the baseline noise shows that the *y*-axis scalings for the three SIM chromatograms are not necessarily the same and that the small peak in the control is still $\ll$1.0 ppb. The control and recovery SIM chromatograms are scaled relative to the 0.10 ng injected SIM chromatogram. Representative SIM chromatograms of 0.10 ng standards, controls, and procedural recovery samples for G-30033, G-28279, and G-28273 are shown in Figures 4, 5, and 6, respectively. The nanograms injected and their respective responses for the target ions for each analyte were used for construction of the calibration plots and quantification. Good linearity was obtained for all four analytes as demonstrated by correlation coefficients $\geq$0.995 throughout the method validation. The limits for analyte confirmation were established by calculating $\pm$20% of the Q1/Tgt ion ratio as measured for an analytical standard. Thus, the Q1/Tgt ion ratio in a sample must be within $\pm$20% of the value calculated for the standard to confirm the identity of an unknown residue. None of the small peaks shown in the control samples in Figures 3B−6B satisfied the Q1/Tgt ion ratio required for analyte confirmation. This was not unexpected since the control urine samples were obtained from known atrazine-exposure-free individuals and accurate integration of the Q1 and target ions at these low concentration levels was not possible due to the urine matrix interferences, which matched the Q1 and/or target ion *m*/*z* and retention time of the analyte. Thus, analyte confirmation was not possible at these low concentration levels ($\ll$1.0 ppb). Consistent analyte confirmation was obtained for samples containing residues and recovery samples at and above the LOQ of the method.

Shown in Table 3 are the individual procedural recovery results for each analyte obtained during the method validation. The fortification levels ranged from

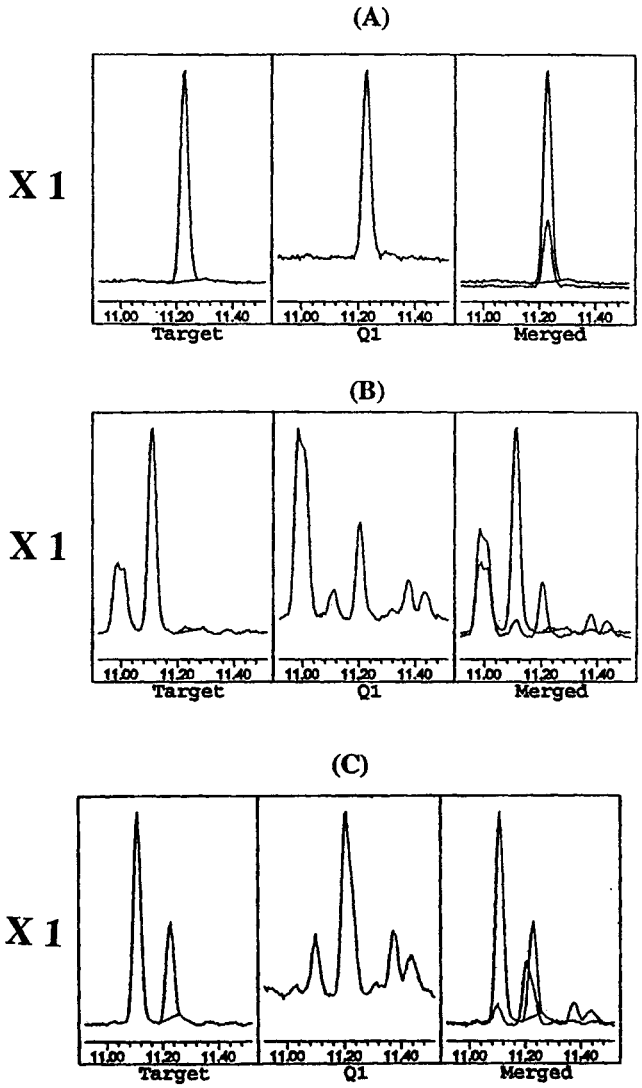


Figure 5. Representative SIM chromatograms for G-28279: (A) 0.10 ng injected standard, (B) control, and (C) 1.0 ppb procedural recovery.

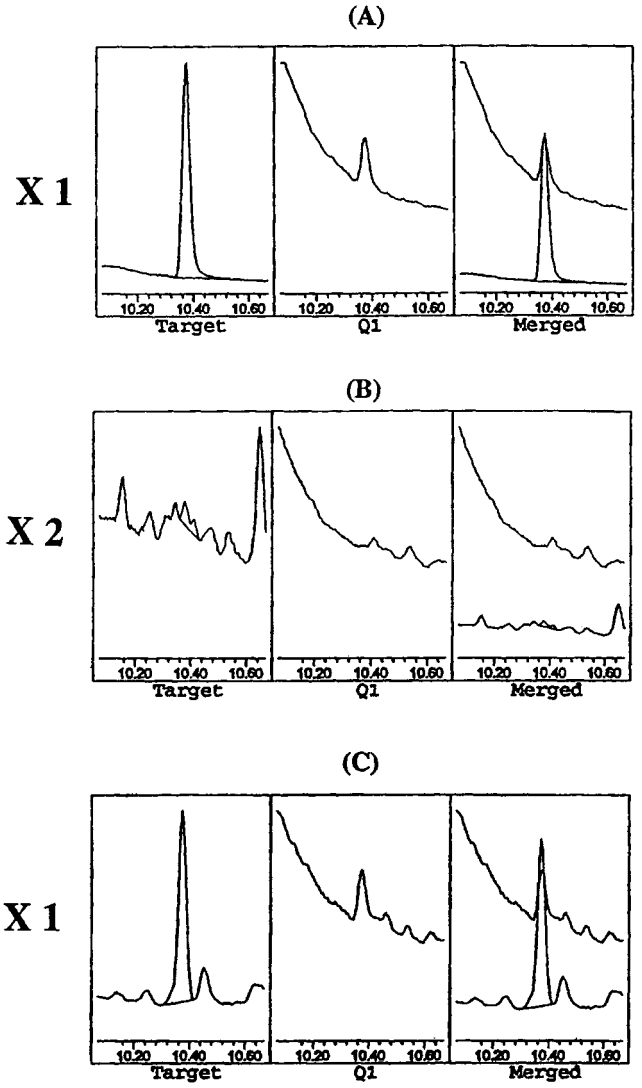


Figure 6. Representative SIM chromatograms for G-28273: (A) 0.10 ng injected standard, (B) control, and (C) 1.0 ppb procedural recovery.

1.0 ppb for atrazine and G-30033 or 2.0 ppb for G-28279 and G-28273 to 200 ppb for each analyte. The mean recoveries and standard deviations were 115, 113, 112, and 97% and 10.6, 10.2, 9.5, and 16.1% for atrazine, G-30033, G-28279, and G-28273, respectively, meeting the U.S. EPA acceptability guidelines of 70−120%. The ranges of recovery values obtained were 87−134, 87−134, 88−137, and 63−131 for atrazine, G-30033, G-28279, and G-28273, respectively. The mean recovery and standard deviation data demonstrate the accuracy and precision of the method, respectively. There is a consistent positive bias in the recovery data for three of the analytes, but this is still well within the acceptance limits established by the U.S. EPA. All reagent blank and control urine samples were ≪1.0 ppb for atrazine and G-30033 and ≪2.0 ppb for G-28279 and G-28273.

Urine is a complex matrix consisting of various amino acids, peptides, proteins, urea, uric acid, creatinine, creatine, sugars, lipids and fats, salts, and numerous other components. Its pH is typically between 4.8 and 7.6, averaging 6.0 (Bray, 1962). The purification procedures used in Method AG-637 were developed to remove as many of these components as possible prior to analysis using GC/MSD. Even under SIM mode instrumental operating conditions, low concentration level sample matrix components are sometimes observed that have the same target ion *m/z* and nearly the same retention time as the analytes. This indicates structural similarities between the analytes and some of the components that comprise the urine. Thus, acetonitrile was added to the urine sample to precipitate proteins and similar high molecular weight components, and Celite was added to assist the filtration process. This fraction was then acidified and subjected to a SAX SPE step to remove those components that were anionic at pH 3.0. Under these conditions, the protonated triazines are cationic, do not interact with the quaternary ammonium functionality of the SAX SPE column, and pass virtually unaffected through the column. This fraction was then basified and subjected to liquid/liquid partitioning with ethyl acetate to remove salts and acidic components. Finally, the sample was subjected to silica SPE purification to remove nonpolar sample components and those highly polar components that were irreversibly adsorbed to the silica compared to the elution strength of the silica column eluent. The relatively "clean" reagent blank and control urine samples plus the monitoring of selected fragment ions obtained during electron ionization of the analytes

Table 3. Procedural Recovery Data for Atrazine, G-30033, G-28279, and G-28273 Obtained during the GLP Method Validation[a]

fortification level (ppb)	atrazine	G-30033	G-28279	G-28273
1.0/2.0[b]	114	117	101	63
1.0/2.0[b]	114	111	113	80
1.0/2.0[b]	120	108	116	92
1.0/2.0[b]	111	111	102	113
3.0	117	94	102	88
3.0	91	107	110	103
3.0	112	108	103	69
3.0	117	110	108	78
4.0	116	113	118	83
4.0	122	119	119	90
4.0	122	119	112	107
4.0	125	122	119	110
5.0	119	119	112	112
5.0	119	118	111	109
5.0	113	118	114	104
5.0	123	111	118	101
20	132	133	125	114
20	108	109	107	85
20	134	134	137	131
20	87	87	88	86
200	112	120	112	98
200	104	115	106	107
200	117	110	118	108
200	111	105	110	105
mean	115	113	112	97
SD	10.6	10.2	9.5	16.1
n	24	24	24	24
max	134	134	137	131
min	87	87	88	63
rel SD	9.2	9.0	8.5	16.6

[a] All reagent blanks and controls contained <1.0 ppb of atrazine and G-30033 and <2.0 ppb of G-28279 and G-28273. All recoveries were corrected for control residues. [b] Atrazine and G-30033 were fortified at the 1.0 ppb and G-28279 and G-28273 at the 2.0 ppb concentration levels.

collectively contribute to the high degree of specificity of the method. These results clearly demonstrate the validity of the sample preparation procedure to eliminate urine matrix GC/MSD interferences and to obtain acceptable recoveries at the method LOQ.

Additional recovery data were obtained during the analyses of urine samples for one of the worker exposure studies, and these results are summarized in Table 4. The mean recoveries and standard deviations were 106, 104, 107, and 95% and 13, 15, 15, and 16% for atrazine, G-30033, G-28279, and G-28273, respectively. These results are comparable to the recovery data obtained during the GLP method validation. A second worker exposure study is still in progress.

The methodology is very rugged in the hands of experienced analysts. Analytical method AG-637 passed a ruggedness trial, conducted under GLP, when tested by an outside contract laboratory (Schuster, 1996). The mean recoveries and standard deviations were 89, 89, 100, and 80% and 7.5, 7.4, 7.9, and 8.6% for atrazine, G-30033, G-28279, and G-28273, respectively.

The limits of detection of the method, defined as the lowest concentration of standard injected used for construction of the calibration plot, are 0.05 ng for G-28273 and 0.025 ng for the other three analytes. The signal-to-noise ratio at the lowest concentration for each analyte is >10. The LOQ of the method, defined as the lowest procedural recovery sample concentration tested in the validation, are 1.0 ppb for atrazine and G-30033 and 2.0 ppb for G-28279 and G-28273.

Table 4. Mean Procedural Recovery Data for Atrazine, G-30033, G-28279, and G-28273 at Each Fortification Level Obtained during the Analysis of Urine Samples from a Worker Exposure Study

fortification level (ppb)	atrazine	G-30033	G-28279	G-28273
1.0/2.0[b]	107	104	109	93
SD	12	16	16	17
n	33	34	33	33
5.0	105	104	105	98
SD	14	15	13	14
n	20	20	20	20
500	91	95	96	96
SD	5	4	2	6
n	2	2	2	2
overall	106	104	107	95
SD	13	15	15	16
n	55	56	55	55

[a] All reagent blanks and controls contained <1.0 ppb of atrazine and G-30033 and <2.0 ppb of G-28279 and G-28273. All recoveries were corrected for control residues. [b] Atrazine and G-30033 were fortified at the 1.0 ppb and G-28279 and G-28273 were fortified at the 2.0 ppb concentration levels

Conclusions. The results presented in this paper demonstrate that Novartis Analytical Method AG-637, validated according to FIFRA GLP 40 CFR Part 160 standards, is valid, accurate, precise, and specific for the determination of atrazine and its dealkylated chlorotriazine metabolites in urine. The method also passed a ruggedness trial by an outside contract laboratory as per the ruggedness testing guidelines required by the U.S. EPA. The method has LOQ of 1.0 ppb for atrazine and G-30033 and 2.0 ppb for G-28279 and G-28273. Novartis Analytical Method AG-637 was submitted to the U.S. EPA as part of a special review for atrazine (Atrazine/Simazine, 1996).

ACKNOWLEDGMENT

We gratefully acknowledge the technical assistance of Amanda L. Bray during method development and the analytical assistance of Laura L. Speer and Jennifer E. Yates during the worker exposure studies.

LITERATURE CITED

Atrazine/Simazine. Additional Information in Response to the United States Environmental Protection Agency's Position Document 1: Initiation of Special Review Nov 23, 1994, Supplement II, Public Docket OPP-30000−60, Oct 31, 1996.

Bradway, D. E.; Moseman, R. F. Determination of Urinary Residue Levels of the N-Dealkyl Metabolites of Triazine Herbicides. *J. Agric. Food Chem.* **1982**, *30*, 244−247.

Bray, W. E. *Bray's Clinical Laboratory Methods*, 6th ed.; Bauer, J. D., Toro, G., Ackerman, P. G., Eds.; C. V. Mosby Co.: St. Louis, 1962; p 557.

Catenacci, G.; Barbieri, F.; Bersani, M.; Ferioli, A.; Cottica, D.; Maroni, M. Biological Monitoring of Human Exposure to Atrazine. *Toxicol. Lett.* **1993**, *69*, 217−222.

Dean, John A.; Composition and pH Values of Buffer Solutions. In *Lange's Handbook of Chemistry*, 12th ed.; McGraw-Hill: New York, 1979; pp 5−77.

Esser, H. O.; Dupuis, G.; Ebert, E.; Vogel, C.; Macro, G. J. Herbicides Chemistry, Degradation, and Mode of Action. In *s-Triazines*, 2nd ed.; Kearney, P. C., Kaufman, D. D., Eds.; Dekker: New York, 1975; Vol. 1, pp 129−208.

Jenke, D. R. Chromatographic Method Validation: A Review of Current Practices and Procedures. I. General Concepts and Guidelines. *J. Liq. Chromatogr. Relat. Technol.* **1996a**, *19* (5), 719−736.

Jenke, D. R.; Chromatographic Method Validation: A Review of Current Practices and Procedures. II. Guidelines for Primary Validation Parameters. *J. Liq. Chromatogr. Relat. Technol.* **1996b**, *19* (5), 737−757.

Jenke, D. R. Chromatographic Method Validation: A Review of Current Practices and Procedures. III. Ruggedness, Revalidation, and System Suitability, *J. Liq. Chromatogr. Relat. Technol.* **1996c**, *19* (12), 1873−1891.

Kumazawa, T.; Sato, K.; Seno, H.; Suzuki, O. Rapid Isolation with SEP−PAK C-18 Cartridges and Capillary Gas Chromatography of Triazine Herbicides in Human Body Fluids. *Forensic Sci. Int.* **1992**, *54*, 159−166.

Lucas, A. D.; Jones, A. D.; Goodrow, M. H.; Saiz, S. G.; Blewitt, C.; Seiber, J. N.; Hammock, B. D. Determination of Atrazine Metabolites in Human Urine: Development of a Biomarker of Exposure. *Chem. Res. Toxicol.* **1993**, *6* (1), 107−116.

Selman, F. B.; Rosenheck, L.; Bodalbhai, L. H.; Brady, J. F.; Cheung, M. W.; Yokley, R. A. Evaluation of the Potential Exposure of Workers to Atrazine During Commercial Mixing, Loading, and Spray Application to Corn. Protocol Number 178-95; Novartis Crop Protection Report ABR-95133 (MRID 44152109), Oct 1996 (unpublished results).

Schuster, L. L. Independent Laboratory Confirmation of Novartis Analytical Method AG-637. ABC Laboratories; Novartis Study 281−96; ABC/CA Study 96606, Dec 1996 (unpublished results).

Shimabukuro, R. H.; Lamoureux, G. L.; Frear, D. S.; Bakke, J. E. In *Metabolism of s-Triazines and Its Significance in Biological Systems*; Tahori, A. S., Ed.; Pesticide Terminal Residues (Supplement to Pure and Applied Chemistry); Butterworth: London, 1971; pp 323−342.

Yokley, R. A., Bodalbhai, L. H., Cheung, M. W. Storage Stability of Atrazine, G-30033, G-28279, and G-28273 in Urine Under Freezer and Refrigerator Storage Conditions. Protocol No. 539-95; Novartis Crop Protection Report ABR-96042, 1996 (unpublished results).

Received for review May 14, 1997. Revised manuscript received October 3, 1997. Accepted October 9, 1997.[⊗]

JF970397Z

[⊗] Abstract published in *Advance ACS Abstracts*, December 15, 1997.